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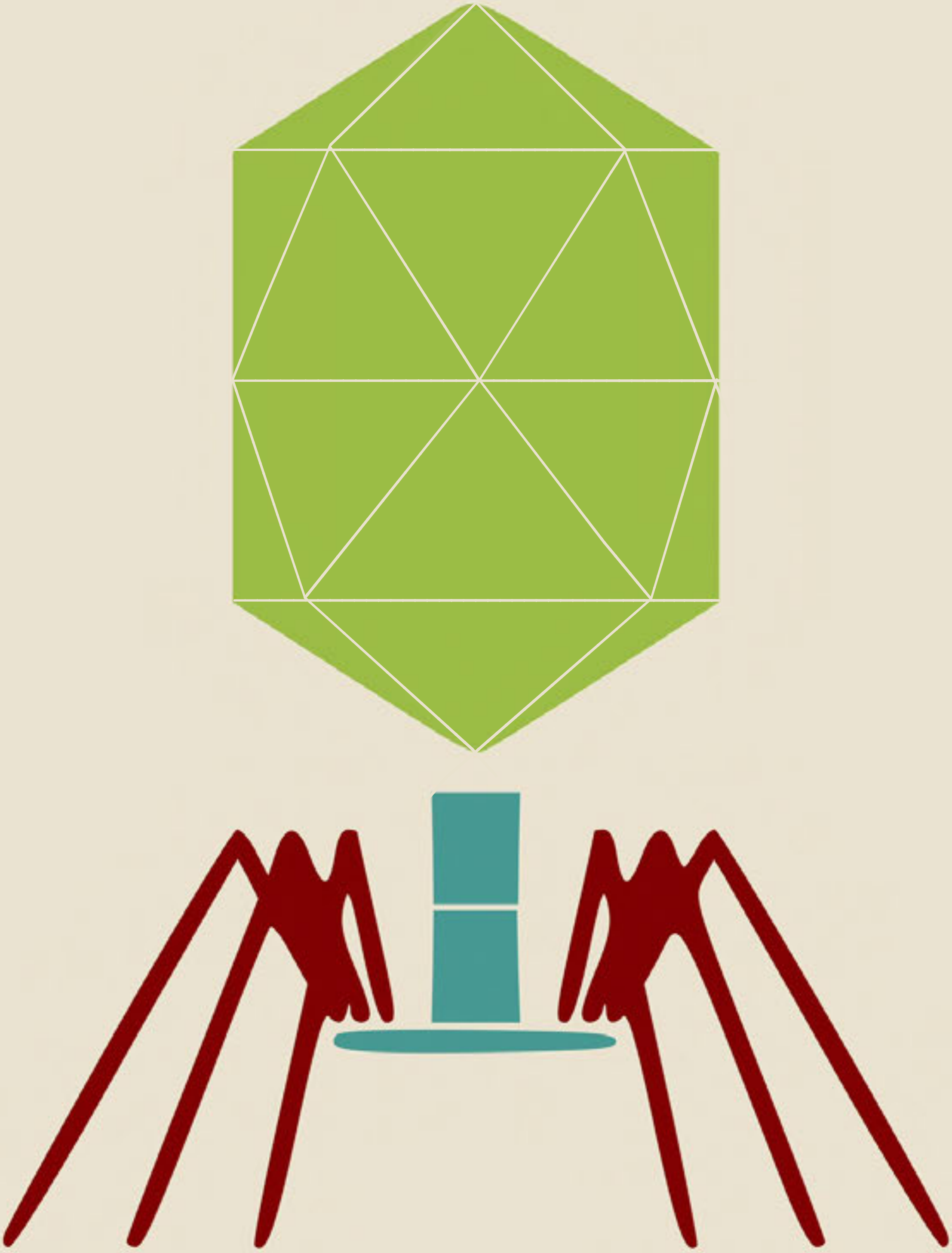
MICROBIOLOGY PURE

Based On Marrow 4.0 Videos

BACTERIOLOGY



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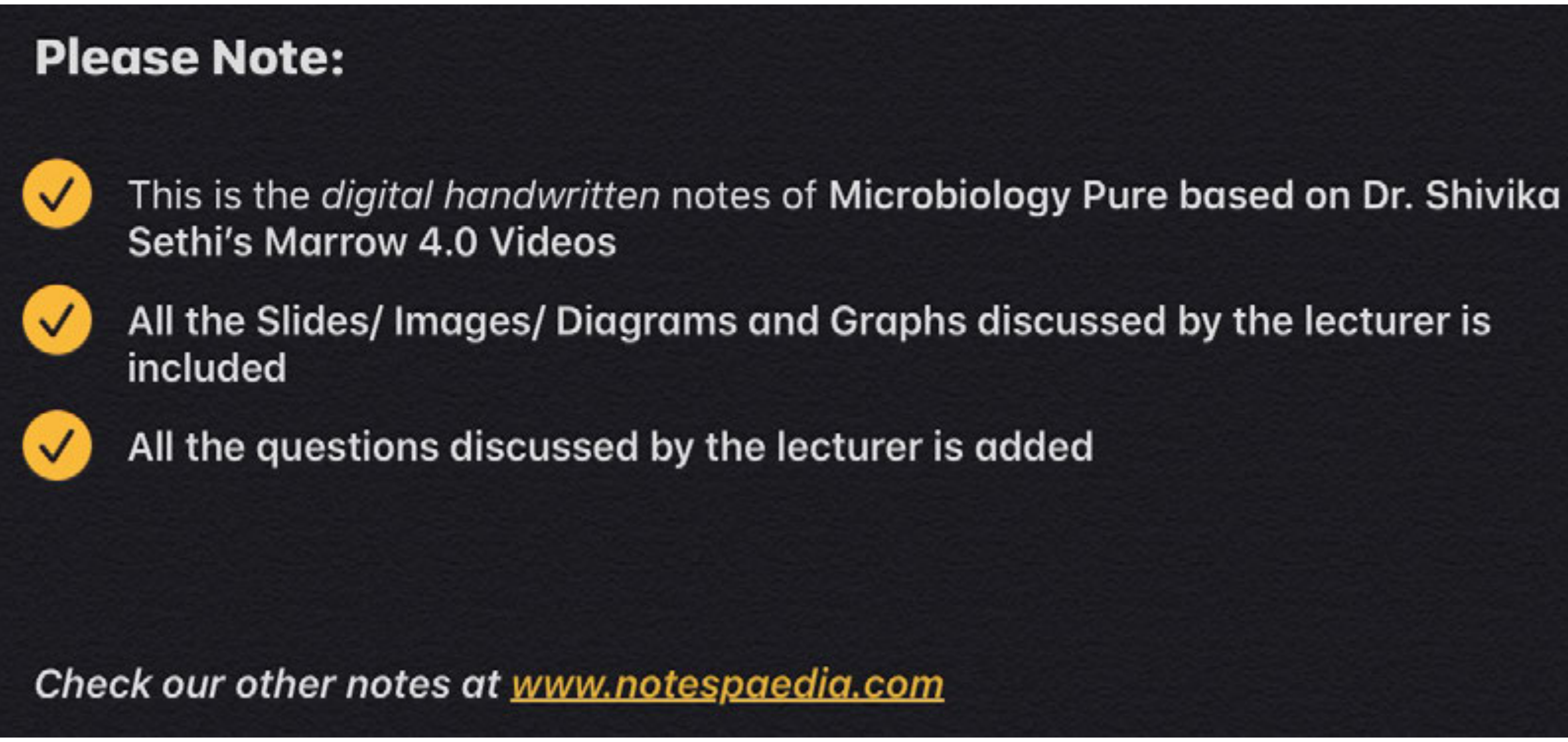

Please Note:

- ✓ This is the *digital handwritten* notes of **Microbiology Pure based on Dr. Shivika Sethi's Marrow 4.0 Videos**
- ✓ **All the Slides/ Images/ Diagrams and Graphs discussed by the lecturer is included**
- ✓ **All the questions discussed by the lecturer is added**

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Table Of Contents - BACTERIOLOGY

SL No.	Chapter	Page No
1	Sterilization & Disinfection - Physical	2
2	Sterilization & Disinfection - Chemical	5
3	Microscopy and Staining	7
4	Cultivation and Antimicrobial Susceptibility	11
5	Bacterial Cell Structure	17
6	Gene Transfer	23
7	Staphylococcus	26
8	Beta Haemolytic Streptococcus	34
9	Alpha and Gamma Haemolytic Streptococcus	37
10	Corynebacterium	40
11	Bacillus	44
12	Clostridium Perfingens and Tetani	47
13	Clostridium Botulinum and Difficile	52
14	Enterobacteriaceae 1	59
15	Enterobacteriaceae 2	64
16	Vibrio and Pseudomonas	69
17	Neisseria	74
18	Treponema	80
19	Chlamydia	88
20	Rickettsia and Related Genera	91

BACTERIOLOGY

sterilisation AND disinfection: physical

Sterilisation
destruction of all forms of life including spore (absolute pheno)

Disinfection
destruction of only the pathogenic microorganisms.

Antiseptics
destruction of living organisms.

Order of Susceptibility

Prions → Bacterial spores → Cysts of protozoa
(most resistant)
↓
Mycobacteria
↓
Non-enveloped virus
↓
Fungi
↓
Gram +ve Bacteri → Gram -ve Bacteria → Enveloped viruses
(most easy to kill)

PHYSICAL METHODS

- ✓ Heat (most effective)
- ✓ Radiation
- ✓ Filtaration

(A) HEAT

(i) Dry heat

MOA : kills by Oxidative damage
Denaturation of proteins
↑electrolyte levels to toxic levels.

Eg : 1. Flaming
Disinfection of mouth of test tubes, core slips, slides.

Eg : 2. Red Heat
Sterilisation of inoculating loops straight wires, tips of forceps

Eg : 3. Incineration

- Sterilisation & Reduction In volume of infectious hospital waste disposal.
- Reductionin volume ~ 80-85%

We sterilise :

- animal carcasses
- human pathological material
- soiled beddings & dressings
- Cytotoxic drugs.

In an incinerator : 2 chambers

(i) Primary Chamber : 650-750°C
(ii) Secondary Chamber : 1050-1100°C

Eg : 4. Hot air oven

Sterilisation of
- all metalic instrument
- glass wares
- oil ; jellies ; powders ; waxes
- cotton swabs.

Temperatures -
- 160°C for 2hrs (MC)
- 170°C for 1hr
- 180°C for 30min

Precautions
- No rubber objects
- No over loading
- cool it for 2hrs before opening.

Efficasy of hot air oven :

Physical : Temperature chart recorder
Chemical : Browne's tube No. 3
(tub c̄ contains a chemical which change colour)
Biological : SPORES OF C.tetani (or) Bacillus subtillis.

(ii) Moist heat (Superior when compared to dry heat)

MOA : Denaturation & coagulation of proteins.

(a) Moist heat - less than 100°C

Eg : 1. Pasteurization : disinfection of milk

- Holder method : moist heat at 63°C x 30min
- Flash method :
  moist heat at 72°C x 15 -20sec → Rapid cooling to temp <13°C
- Ultra high temp (UHT) method : moist heat at 149°C x 0.5 sec

∴ Coxiella Burnetti : can survive in holder method.

Efficacy testing of Pasteurization

(a) Coliform test : (Superior)

Pasteurized milk + MacConkey broth ⇒ Overnight ⇒ No-acid + gas.

(look for the presence of coliform bacteria)

(b) Phosphatase test : MC Used

Pasteurized milk + substrate (disodium phenyl PO_4)

↓ 2hours

No colour change

∴ No colour change → because → Denaturation of Phosphatase Enzyme → Substrate not broken

(c) Serum bath :

moist heat at 60°C for 1 hour for 3 consecutive days.

(d) Vaccine bath :

moist heat at 60°C for 1 hour for 3 consecutive days

(e) Inspissation :

moist heat at 80-85°C for hour for 3 consecutive days.

sterilisation of egg and serum containing media.

Eg : LJ medium, Loeffler's medium.

(b) Moist heat at 100°C

Eg : 1. Tyndallisation / Fractional sterilisation

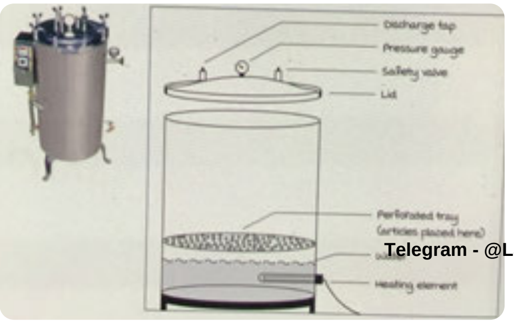
- at 100°C
- for 20 min for 3 consecutive days.

Done in ⟶ KOCH and ARNOLD STERILISER

- Sterilisation of media :- TCBS ; XLD ; DLA ; Selenite F Broth ; Sugar solution ; Gelatin media.

2. Autoclave

- Principle : use of saturated steam under pressure
- MC used : moist heat at 121°C x 15-20 min. (Pressure ⇒ 15psi)
  moist heat at 134°C x 3min (Pressure ⇒ 30 psi)
- Component :



- Uses of autoclave :
  Sterilization : dressings ; linen ;
  Non sharp metallic instruments
  Aqueous solution ;
  Microbiological media
  Plastic pipette tubes

- Efficasy testing of Autoclave :
  - Physical : Temparture chart recordor
  - Chemical : Browne's tube No : 1; Bowie dick tapes
  - Biological : Spores of Bacillus stearothermophillus

(B) RADIATION

(i) Non ionizing - UV Rays.

- UV Rays : λ of 220-280 nm
- MOA : DNA damage
- Poor penetrating power
- Non-sporicidal
- Disinfection of biosafety cabinets; hospital corridors.

✓ Efficasy testing → Spores of B. pumilus.

(ii) Radiation - Ionizing :

- γ-rays → cold sterilization
- MOA : DNA damage
- High penetrating power
- Sporicidal
- Used for sterilization of
  - Disposable: Gloves
Petriplate
Syringes.
  - IV sets; foley's catheters
  - sutures; Implants; Prosthesis
  - Glass wares
  - Fabrics; contraceptive devices; cotton swabs

(C) FILTRATION

- for heat sensitive liquids

Eg : Sera; Antibiot solutions; vaccines; sugur/urea solution; Gelatin media

Poor size of standard Bacterial filters ⇒ 0.45μ

Efficacy testing : Serratia marcescens
Brevidimonas diminuta.

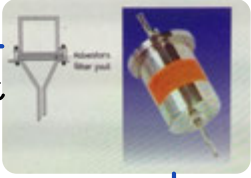
Types :

(a) Earthern wave filters

- made up of Diatomaceous or procelain earth.
- Shaped in the form of candles

Eg : Pasteur-chamberland filters.
Berkefeld filters
Mandler filters

(b) Asbestos filters :



- made up of magnesium trisilicate
- shape in the form of discs

Eg : Seitz filters

It has carcinogenic potential

(c) Sintered glass filters :

- shaped in the form of discs
- Disadvantage : Expensive

(d) Membrane filters : (millipore)

- made up of cellulose esters; polyester
- available in the form of discs
- single use discs
- MC : Used filters

sterilisation AND disinfection: chemical

FDA CLASSIFICATION

	Bacteria	Fungi	Virus	Mycobacterium	Spores	Groups
High level	+	+	+	+	+	Ethylene Oxide, Plasma, Aldehyde
Intermediate Level	+	+	+	+	+/−	Phenols & Halogens.
Low level	+	+	+/−	+/−	−	Alcohols, Surface Acting agents.

ALCOHOLS

✓ MOA: Coagulation of proteins; dehydration of cells

✓ 60-80% concentration used

ETHYL ALCOHOL → Spirit

ISOPROPYL ALCOHOL → Hand disinfectant

METHYL ALCOHOL → killing fungal spore in Biosafety cabinets.

HALOGENS

✓ MOA: Oxidation of sulf-hydryl residues of proteins

CHLORINE COMPOUNDS

- Disinfection of water supplies } Cl_2 tablets
- Sodium Hypochlorite
  - disinfection of infectious hospital spills
  - Hospital floor

IODINE COMPOUNDS → Skin antiseptics

a) Tincture I_2 ⇒ 2% iodine in 70% alcohol

b) Betadine ⇒ I_2 coated onto a neutral carrier Polyvinyl pyrrolidone.

hence also called as Povidone iodine

SURFACE ACTING AGENTS

- Damaging membranes: MOA

Cationic → Cetrimonium bromide (SAVLON)

Anionics → Soaps

Savlon → Chlorhexidine + Cetrimide

Dettol → 10% Chlorxylenol

ALDEHYDES

✓ MOA: Alkylation of hydroxyl, amino & carboxyl residue of proteins

FORMALDEHYDE (40%)

(i) fumigation of OTs; wards etc.

(ii) DUCKERING → process of killing B. antracis spores.

(iii) Preservation of Anatomical specimen.

GLUTERALDEHYDE/CIDEX - 2%

(i) Sterilisation & disinfection of anaesthetic equipment.

(ii) Cysto/broncho/endoscopes corrugated rubber tubings endotracheal tubes.

- Sterilisation (6-8 hours)
- Disinfection (25-30 min)
- Reused for (10-14 days)

ORTHO-PHTHALDEHYDE (OPA) - 0.55%

- same use as cidex
- active at wide pH (Cidex only at alkaline pH)
- Less irritant
- Better mycobacterial activity.

PHENOLICS - COAL TAR DERIVATIVE

MOA: Precipitation of proteins
DNA Damage.

5% Phenol
1-4% Cresol
2-5% Lysol

- surface distention of hosp floor.
- Discard jars
- Active in organic matter

Chlorinated biphenolics (modified phenolics)
↳ least corrosiveness

- Chlorhexidine - Antisepsis & Hand disinfectant
- Hexachlorophene
- Chlorxylenol
- Triclosan

ETHYELE OXIDE/ETO GAS

- 9% ETO + 91% CO_2 or Hydrochloroflurocarbon
- Sterilization: Heat labile & moisture sensitive objects.
    - Beddings, Blankets; rubber & plastic objects
    - Disposables; Heart lung machines; respirator; dental equipment.
- 1 Cycle time of ETO Sterilization → 18-24 hrs (Aeration required) because ETO is very toxic gas
- 2 types of ETO Sterilizer: Cold Cycle ⇒ 37-44°C
  Hot Cycle ⇒ 55°C
- Efficasy :→ spores of bacillus globigus (pigmented variant of B. subtilis)

Disadvantage:
→ Toxic: Exposure monitoring needed because of Cataract & neural disturbance.
→ Carcinogenic
→ Irritant
→ Inflammable.

PLASMA (VHP) STERILIZATION

✓ VHP - Vapourised H_2O_2
✓ 59% H_2O_2 Gas.
1st step : Creation of vaccum
2nd step : Introduce gas
3rd step :- Electro magnetic radiation
Radiowaves. Microwaves

- Releases → toxic O_2 radicals
(interfere the metabolism of organisms)

PLASMA STERILIZER (STERRADS):
- Temperature : 30-60°C
- Cycle time : 60-75 min.
- Efficasy : Spores of B. Stearothermophillus
- Use : Sterilization of heat sensitive metal (or) non-metal hospital devices.

[∴ Plasma sterilizer should be dry]

SPAULDING CLASSIFICATION

✓ Dr E.H Spaulding divides into categories based on the risk of infection involved with their use.
✓ There are 3 groups

(a) Critical:
- enter sterile tissues of body & vasculature of body
- "sterilized" before use.

Eg: Surgical instruments; implants; prosthesis; catheters; laproscopes; Foley's catheter

(b) Semicritical:
- contacts with mucous membrane & breaks in skin

Eg: GI Endoscopes
Bronchoscopes
Cytoscopes
Anaesthetic equipment
} Sterilised or high levels of infection

Oral & Rectal thermometers → Intermediate level of distection

Notespaedia.com More than just notes...

(c) Non-critical
- contact with intact skin

Eg: Stethoscope
ECG Electrode
Bed pans

EFFICASY OF A DISINFECTANT

(1) Phenol co-efficent : Efficasy compared with that of phenol
- serial dilution of the new disinfects & also that of with phenol

↓

each of these dilutions add standard Inoculum of S.typhi or S.aureus

↓

after every 5min we will subculture into a solid medium.

↓

we will find out highest dilution of the new disinfectant as well as of phenol which kills organism in 10min ; but not in 5min

dilutions will be calculated respectevely

determine ratio

finally we find out the phenol-coefficient

✓ if phenol-coefficient >1 ⇒ > efficasious than phenol
phenol-coefficient <1 ⇒ < efficasious than phenol
phenol-coefficient = 1 ⇒ Equal efficasious

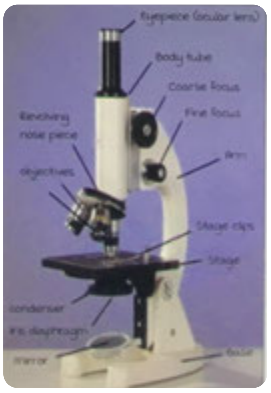
TESTS :
1. Rideal Walker Test
2. Chick martin Test → better ; superior test

↓

Because it's more realistic ; along with Staph.aureus ; S.typhi, we also add 3% sterile feces in this test

microscopy AND staining

LIGHT MICROSCOPE / OPTICAL MICROSCOPE



✓ Leeuwenhook introduced simple microscope
✓ also known as Bright field microscope
✓ Jansen → introduced Compound microscope.
✓ wavelength of light used → 500-550nm.
✓ Condenser : focuses light on stage.
✓ transmited light through the structure of stage pases through

(1) Objective lens : mounted on revolving nose

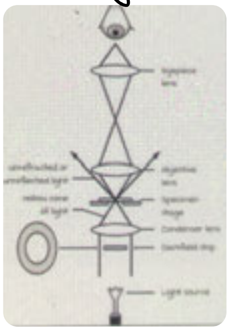
Scanner lens	→	4X
Low dry objective lens	→	10X
High dry objective lens	→	40X
Oil immersion lens.	→	100X

(2) Eye piece/ocular lens : → 10 x

FINAL MAGNIFICATION = EYE PIECE × OBJECTIVE LENS

✓ Resolution of light microscope : 0.2 - 0.3 μ

DARK FIELD MICROSCOPY

- Used for observing slender bacteria
  Eg: Spirochaetes
- 

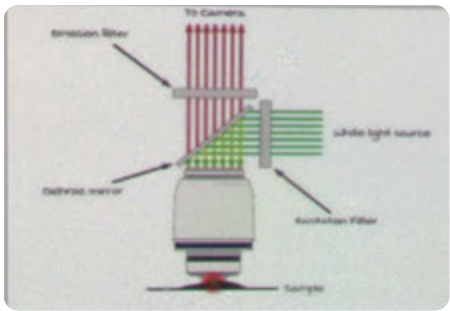
- condensing lens has a central stop
  ↓
  Object illumination by hollow cone of light
  ↓
  Reflected light reaches the observer's eye.

FLUORESCENCE MICROSCOPE

- Fluorochromes (fluorescent dyes are used)

Absorbs short wavelength (uv) → Fluorochromes
↓ Emits
Visible lights
longer wavelength

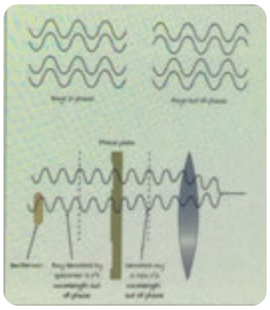
- Fluorescent dyes
  - Auramine O } stains mycolic acid
  - Rhodamine B }
  - Acridine orange → ss/ds DNA
  - FITC - Fluorescence isothio Cyanate } Binds to Ab
  - Lissamine Rhodamine }



- Whight light source → uv light
- Excitation filter : will allow only shorter wavelength to pass through
- Shorter λ falls on the object
  ↓
  emits longer λ
- Dichroic mirror → Allow only longer λ light

PHASE CONTRAST MICROSCOPY

✓ used for unstained specimens
✓ visualisation of live organisms.
✓ High contrast image
✓ Chances in phase of light
↓
Phases plate.
✓ Results in change in intensity/Amplitude.

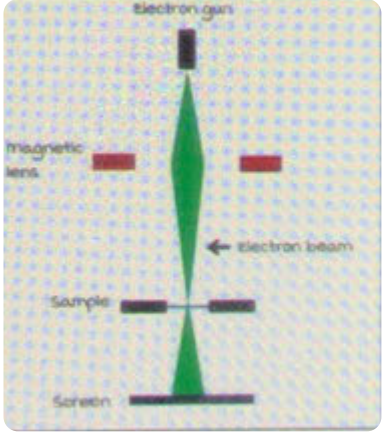


ELECTRON MICROSCOPE

✓ Introdused by Knoll & Ruska
✓ Electron gun → generate e⁻ of λ (0.005 nm)
✓ It has "vaccum"
✓ Electromagnents → focuses the electron

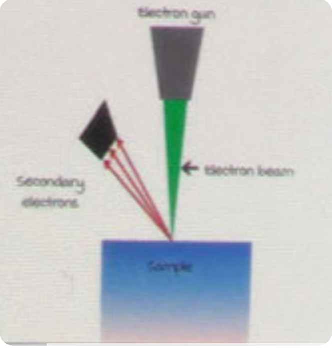
Transmission EM :

- to visualise internal structures
- Transmitted electrons are recieved on a fluorescent viewing screen.



Scanning EM :

- to visualise Surface structures
- back scattered electrons are recieved on screen



STAINS

(1) Simple staining
Eg : Methylene blue

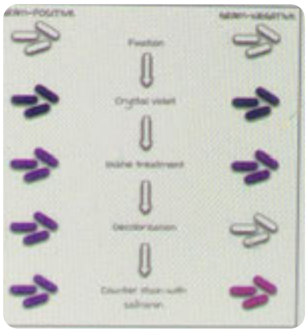
2) Differential staining
Eg: Gram stain
Acid fast stain

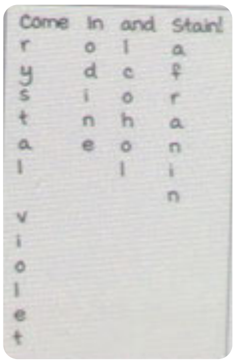
GRAM STAIN ;

✓ Introduced by Hans Christian Gram

4 Steps:

(1) 1° stain → Crystal violet/ methyl violet/ Gentian violet
(2) Mordent → Iodine
(3) Decolouriser → Alcohol/ Acetone & Alcohol - Acetone
(4) Counter stain → SAFFRANINE / BASIC FUSCHIN/Neutral Red





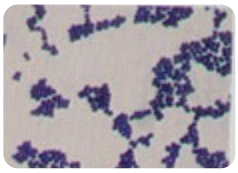
Come	In	and	Stain!
r	o	l	a
y	d	c	f
s	i	o	r
t	n	h	a
a	e	o	n
l		l	i
			n
v			
i			
o			
l			
e			
t			

✓ Gram +ve Cytoplasm → more acidic → Binds basic stain → 1° stain better
✓ Gram +ve Cell wall is thicker → so retains 1° stain better
✓ Gram -ve cell wall → more lipids → Get dissolved by Alcohol /Acetone
↓
leads to pore formation

MORPHOLOGY OF BACTERIAS.

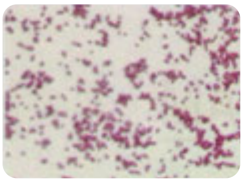
Gram positive cocci

✓ Staphylococci
✓ Streptococcus
✓ Enterococcus
✓ Micrococcus



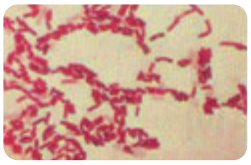
Gram negative cocci

✓ Neisseria
✓ Moraxella
✓ Vellionella



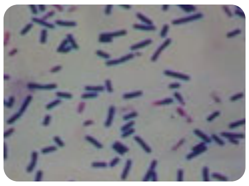
Gram negative rods (Bacilli)

✓ Enterobacteriacea
✓ Pseudomonas
✓ Vibrio (curved)
✓ Prevotella
✓ Porphyromonas
✓ Legionella
✓ Bacteroides



Gram positive rods (Bacilli)

✓ Corynbacterium
✓ Mycobacterium



✓ Bacillus } SPORE
✓ Clostridium } Forming
↳ Anaerobes

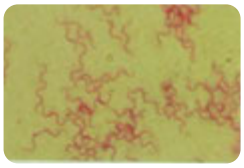
✓ Actinomyces } → Anaerobes
✓ Nocardia } Branching Rods

✓ Bifidobacterium } Anaerobes
✓ Eubacterium }

- Listeria
- Lactobacillus → Anaerobes

Gram negative spirals

✓ Spirochetes
✓ Campylobacter
✓ Helicobacter
✓ Spirillum



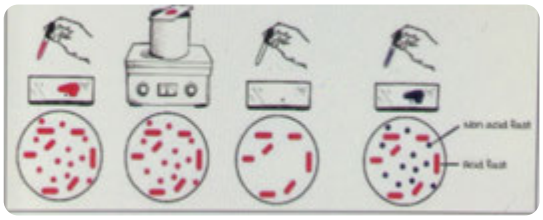
Gram negative Coccobacilli

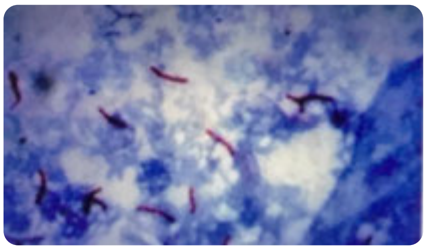
✓ Brucella
✓ Bordtella
✓ Hemophillus
✓ Franciella
✓ Chlamydia
✓ Ricketssia
✓ Acenetobacter
✓ Pasturella.

ACID FAST STAIN

- ✓ 1st introduced by Paul Ehrlich
- ✓ modified by ZIEHL NEELSON (ZN) Stain

Carbol fuchsin → Decolouriser → Counter stain
(Basic fusin in phenol) 20% H_2SO_4 Methylene Blue
or picric acid
or Malachite green





- Here the Counterstein ↓ Methylene blue
- Acid fast structures → Retain Carbol Fuchsin

Partially acid fast

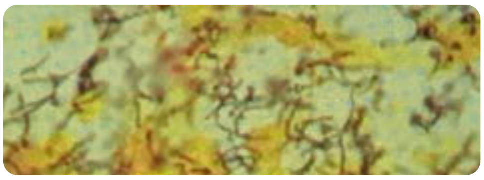
(1) with H_2SO_4 → M. leprae
Oocyst of isospora
cyclo pora
Cryptosporidium

(2) with 0.5-1% H_2SO_4 → Nocardia
Rhodococcus
Legionella micdacei
Taenia saginatea oocyst

(3) with 0.25-0.5% H_2SO_4 → Bacterial spores
Head of sperm

Acid fast (20% H_2SO_4)

- M. tuberculosis complex
- Atypical mycobacteria

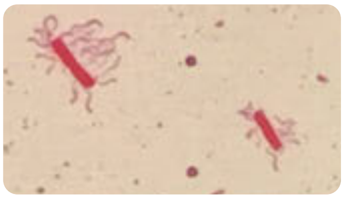


Levaditi stain

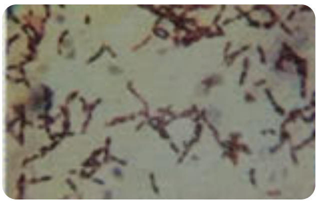
IMPREGNATION STAINS

1. Spirochetes :
↓
Silver impregnation stain
→ Levaditi → for tissue section
→ Fontana → for films

2. Flagella → LEIFSON & RYU Stain

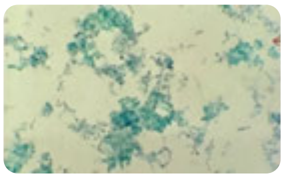


Special Stain



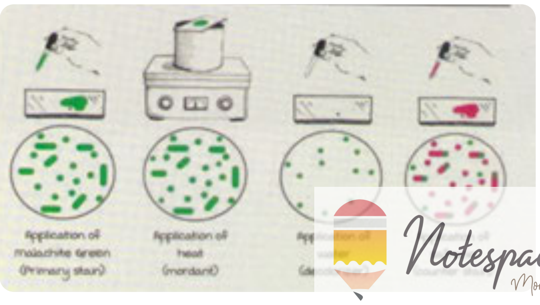
Sudan B Black stain
↓
stains lipid granules
- Polysaccharide granules
↓
Stained by Iodine

VOLUTIN/Metachromatin granules.



- Present in Corynbacterias
- Stained by
  Albert
  Neisser
  Ponder

✓ For spores :
↓
ASHBY Modification of Schaeffer Fulton stain
Reagents used:
SAFFRANINE (Stains the cytoplasm)
+
MALACHITE GREEN (Stains the spore)


Application of malachite Green (Primary stain)
Application of heat (mordant)

cultivation AND antimicrobial susceptibility

CLASSIFACTION OF BACTERIA

Bacteria

- **Phototrophs**
  Derive all the energy from sunlight
- **Chemotrophs.**
  Undergo chemical reactions for synthesis of energy
  - **Autotrophs**
    Utilise atmospheric gases
  - **Hetrotrophs**
    - Require preformed organic compounds
    - most common human commensals & pathogen
    Also known as Chemo organotrophs

CULTURE MEDIUM

- **Louis pasteur** : Established growth requirement of bacteria (Minimal Req: C & N)
  Used Liquid media/broth.
  ↓
  Adv: Rapid growth.
  Disadv: Growth seen as turbidity.
- **Robert Koch** : used solid media
  ↓
  Adv: Identification can be done by colony morphology.
  Disadv: Takes time for colony formation.

SOLIDIFYING AGENTS :-

1. Gelatin

- derived from animal bone & hide
- Protein in nature
- 15% conc
- Disadvantage:
  (1) Proteolysed by several bacteria
  ↓
  Liquefaction of media
  (2) Solid only at temp <24°C

2. Agar-Agar (Chinese grass)

- Derived from sea weeds & Red algae
- Polysaccharide in nature
  - 70% → Agarose
  - 30% → Agropectin
  - Inorganic phosphate + Ca^{2+} salts
- Advantage over Gelatin:
  1) IT'S INERT
     - not hydrolysed by bacteria
     - contains no growth promoters
     - provides no nutrition.
  2) Solid at <42°C & melts at >98°C
     - conc : 1.2 – 2% (MC: 2%)

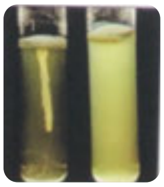
• Soft Agar :

- conc. agar is reduced to 0.2 - 0.5%
- use : to test the molity of bacterium
  ↓
  Hence called Motility Test Agar
  ↓
  - Semisolid in nature
  - Transparent

- stab inoculation of bacteria in to medium

(after few hours)

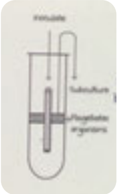
Opaque line of growth → NON MOTILE

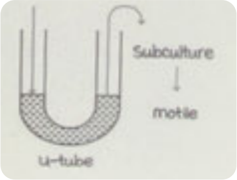


Whole medium becomes translucent → MOTILE

- other method of testing motility using soft agar :

CRAIGIE'S TUBE
↓
Subculture from broad outer tube





U-TUBE
↓
Subculture from the end opposite to site of inoculation

↓

Recovery of colonies
⇓
Organism is motile.

• Firm Agar

- conc increased to 5-6% agar
- hard
- Use : Inhibition of swarming
- Other methods of swarming inhibitions
  - * CLED or MacConkey Agar
    ↳ Cystine lactose Electrolyte deficient
  - * Add → Alcohol, Boric acid, Bile salts, Chloral hydrate, Sulfonamides, Sodium azide, Surface acting agents.
- SWARMING BACTERIAS.

Gram positive
- C. tetani
- B. cereus

Gram Negative
- P. vulgaris
- P. mirabilis
- V. alginolyticum
- V. parahemolyticus

✓ Other solidifying agents :
- Serum
- Eggs.

TYPES OF CULTURE MEDIA

* Simple or Basal media
- Source of C & N
- Nutrionally non fastidious bacterias.
- Eg : Peptone water

Peptone water + meat extract ⟶ Nutrient broth
Peptone water + meat extract + agar ⟶ Nutrient agar

ENRICHED MEDIA

- Nutritionally fastidious bacteria
- Egg, blood, serum added.
- Eg: 1) Blood Agar

  - MC used conc: 5%.
  - 5ml sterile sheep blood
    +
    95ml of autoclaved nutrient agar cooled cooled to 70-75°C

2) Chocolate agar

  - 5 ml of sheep blood
    +
    95ml autoclaved nutrient agar cooled to 70-75°C

3) Egg containing enriched medium:
  - Lowenstein-Jensen medium
  - Dorcet egg medium

  both used for MYCOBACTERIUM

4) Serum containing enriched medium:
  - Loeffler's serum slope → C. diphtheriae
  - 'PPLO' broth / agar → Mycoplasma
    ↳ Pleuropneumonial like organism

ENRICHMENT MEDIUM

it's a liquid selective media

Eg: Alkaline peptone water → Vibrios
Selenite F-broth → Salmonella, shigella.

INDICATOR MEDIUM

- contains indicator agent which gives a charachteristic colour toa group of bacteria
- Eg:

MacConkey Agar	Neutral red
TCBS	Bromothymol blue
Hugh Leifson media	Bromothymol blue

TRANSPORT MEDIUM

- Venketaraman Ramakrishnan (VR) : Vibrio
- Pike's medium : S. pyogenes
- Thioglycolate broth : Anaerobes
- Cary blair : Universal stool transport medium

SELECTIVE MEDIA

- use selective agent →
  inhibit growth of unwated bacteria
  favours growth of wanted bacteria.

(a) Change in pH

Optimum pH for pathogenic bacteria → 7.2-7.4
Optimum pH to vibrio : 8.2-8.4.
Eg: TCBS medium

(b) Adding antibiotics:

- Thayer martin medium (Vancomycin + Colistin + Nystatin) } Neisseria
- Modified Thayer martin (Vancomycin + Colistin + Nystatin + Trimethroprim) } Neisseria
- PPLO medium → Mycoplasma. (Pencillin)

(c) By chemicals:

* Bile salts

MacConkey medium:
- contains sodium taurocholate
- selective medium gram -ve bacteria.

DCA Medium:
- Sodium deoxycholate added.
- Selective for Salmonella, Shigella

(Deoxycholate citrate Agar)

* Potassium tellurity.
- Cornybacteria → resistant to 0.3-0.4% Potassium tellurite

Eg: Tinsadale medium

* 7-10% salt;
- All staph are resistant to this conc of salt
- Eg: Salt Milk agar

(d) by dyes

- Eosin methylene blue agar → selective for gram -ve bacteria
- LJ Medium → selective for Mycobacteria
  ↳ Malachite green

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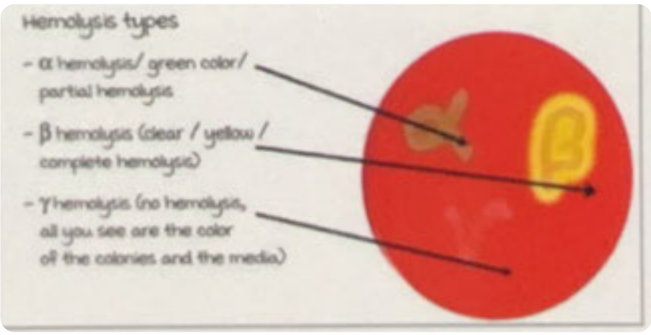
DIFFERENTIAL MEDIUM

✓ Based on the colony morphology / colour, groups of bacteria can be differentiated

Eg : 1) Blood Agar

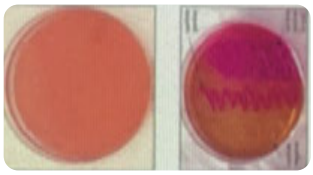

Hemolysis types
- α hemolysis/ green color/ partial hemolysis
- β hemolysis (clear / yellow / complete hemolysis)
- γ hemolysis (no hemolysis, all you see are the color of the colonies and the media)
β

2) MacConkey Agar

Lactose fermenting gram -ve v/s Non lactose fermenting

Lactose fermenting gram -ve ↓ PINK / MAGENTA

Non lactose fermenting ↓ PALE

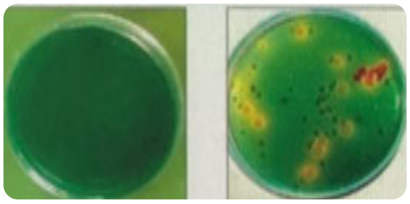


3) TCBS ;

Sugur fermenting Vibrio v/s Non sugur fermenting vibrio

Sugur fermenting Vibrio ↓ Yellow

Non sugur fermenting vibrio ↓ Pale

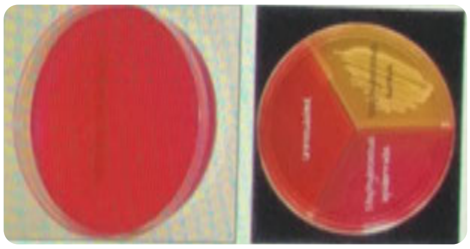


4) Mannitol Salt agar :

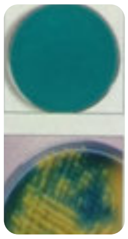
- selective medium for Staphylococci

Mannitol fermenting ↓ YELLOW

Non-manitol fermenting ↓ PALE



5) CLED Medium ;



- Cysteine Lactose Electrolyte Deficient Medium
- Isolate urinary pathogen

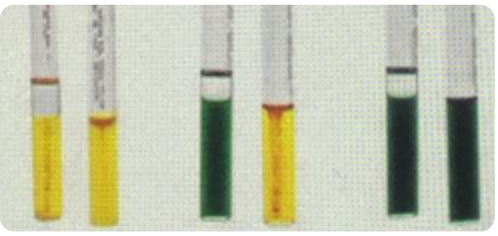
Lactose fermenting ↓ Yellow

Non Lactose fermenting ↓ Pale

6) Hugh Leifson Oxidative Fermenting Medium

- used for differentiating utilisation of Glucose
- if utilisation Aerobic ⇒ Oxidative Utilisation
- if utilisation Aerobic & Anaerobically ⇒ FERMENTATIVE Utilisation
- contains Glucose + Bromo thymol blue (indicator)
- Semisolid media

Evaluation of Hugh-Leifson O-F Method :



Ferm

Oxid ↓ Non fermenters

Not utilised Glucose.

Oxidative Utilisation : Utilise Glucose as C Source + Aerobes

Eg : Pseudomonas Aergoni
Brucella
Bordetella.

Fermentative Utilisation : Eg : E.Coli
Enterobacteriaceae
Staphylococci
Streptococci

Fermentative Utilisation ↓ Utilise Glucose as Source of C + Facultative anaerobes

ANTIBIOTIC SENSITIVITY TEST

- carried out to determine which antibiotic will be most successful in treating a bacterial infection in vivo

Standard inoculum of test bacterium:

- special suspension of test bacterium with specified turbidity, Expressed in the form of Mac Farlaud's. (Unit for expressing the turbidity of bacterial suspension)
- Spectrophotometer: • used for measuring the turbidity of standard inoculum of bacterium
- Temperature for incubation } of antibiotic → 35°C - 37°C
  Time of result interpretation } sensitivity test → 16 - 18 hrs. (overnight incubation)

METHODS OF SENSITIVITY TES

- dilution method
- disc diffusion
- E test / Episilometer test

DILUTION METHOD (Reference method)

- Agar dilution (solid media)
- Broth dilution (Liquid media)
  - Microbroth test (petri plates)
  - Macrobroth test (test tubes)

Broth dilution methods:

-Medium: NUTRIENT BROTH

Eg: UTI → E.Coli → Overnight broth culture in peptone

⇓

Standard inoculum

-In macroboth dilution method, we will take same amount of Nutrient broth in series of test tubes

↓

doubling dilution of antibiotics prepared in nutrient broth

+

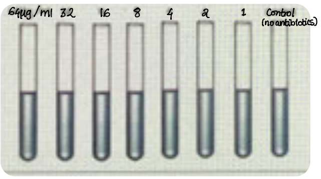
Control with no antibiotics

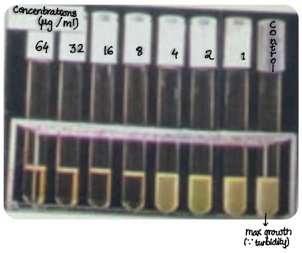
↓

Add 1ml of standard inoculum into all test tubes.

↓

Incubate overnight @37°C





In the above image

- control: max growth (max turbidity)
- As the conc ↑ses (1→4) Turbidity ↓ses.
- in 8μg/ml → no turbidity

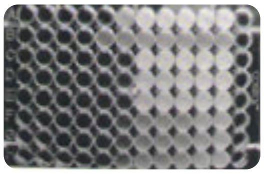
↑

minimum inhibitor conc. (MIC)

Interpretation of Dilution method:

- RESISTANC : MIC > Breakpoint value
- SENSITIVE : MIC < Breakpoint value

if the whole process done in petridishes ⇒ microbroth dilution

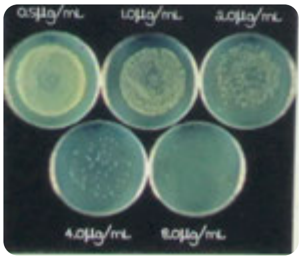


Disadvantage:

- Cumbursome & laborious
- Fastidious organism can't be tested.

Agar dilution method:

- Medium: Cation adjusted muller hinton agar



Prepare serial dilution

↓

Add standard inoculum

↓

Spread it

↓

Look for the lowest conc. of antibiotic that prevent appearance of colonies

- Advantages:
  (a) for fastidious bacterias also (Muller hinton agar + 5% blood)
  (b) For anaerobes (Wilkren Chalgren agar)
- Disadvantage: Cumbersome

DISC DIFFUSION METHOD

Kirby Bauer disc diffussion

- MC method in use
- Medium: Mueller Hinton Agar (5% blood added if required)
- Standard inoculum of 0.5 MCF turbidity

↓

Using a sterile cotton swab, the inoculum is spread onto the MH Agar medium

↓

Lawn culture of bacteria

↓

Discs (impregnated with single standardised conc. of antibiotics) are placed on the swab inoculated petriplates.

↓

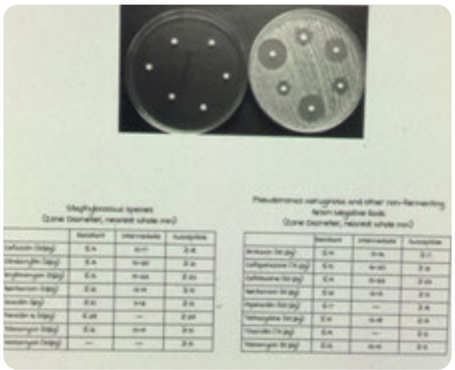
Overnight incubation

↓

Zones of inhibition seen

↓

Measure & compare with standardised tablets



Advantage: very easy to do

Disadvantage: MIC can't be determined.

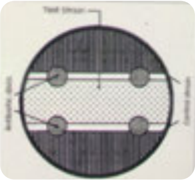
∴ DISC DIFFUSION METHOD is a QUALITATIVE METHOD

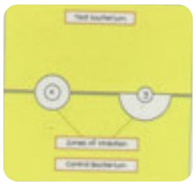
Stoke's method

- only followed in certain Europian countries
- On the same plate →

Antibiotic disc
Control strain } incubated at
Test strain } same conditions

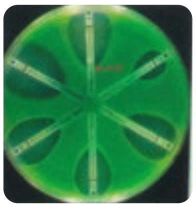
∴ here there is no need of any tables to compare.





- Advantage: Easy to do
- Disadvantage: MIC - not determined.

E-TEST (EPSILOMETER TEST)



- combination of dilution & disc diffusion method
- medium: Muller hinton Agar (+5% blood if required)
- Standard inoculum of of 0.5 MCF turbidity
- Instead of disc, Plastic strips impregnated with graded conc. of antibiotics.
- where the zone of inhibition intersect with plastic strips, that will determine the MIC.
  - Easy to do
  - Quantitative test

Disadvantage: Sometimes result may confuse

↓

go for dilusion/disc diffusion

bacterial cell structure

- Kingdom : Monera (contains unicellular prokaryotes)
- divided in to 2 domains

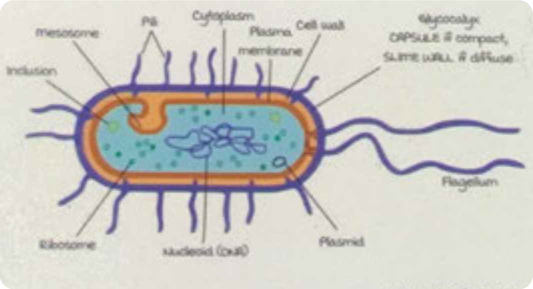
Bacteria → Contains : EUBACTERIA, CYANOBACTERIA

Archae → Contains : Archae bacteria (not pathogenic in human) (grows in extreme conditions)
also called as EXTREMIOPHILES
Eg : Thermus aquaticus

PROKARYOTES VS EUKARYOTES

	PROKARYOTES	EUKARYOTES
1. Nuclear membrane, Nucleolus, histones	Absent	Present
2. Cytoplasmic membrane bound organalles	Absent	Present
3. Chromosome	single, circular	Multiple linear
4. Extrachromosomal DNA in	Plasmids	Mitochondria
5. Mesosomes	Present	Absent
6. Ribosomes	70s ribosome (30s+50s)	80s ⇒ 60s + 40s
7. Cell wall	Muramic acid is present in cell wall	doesn't contain muramic acid if cell wall present
8. Sterols in cell membrane	Absent	Present

Bacterial Cell Structure



- Outer layer in some ⇒ GLYCOCALYX → compact : CAPSULE; diffuse : SLIME
- Cell wall contains muramic acid
- Cell membrane doesnot contain sterols
- Cytoplasm : 70s ribosome
  membrane bound organelles absent
- Mesosomes : invagination of cell membrane
  Site for Respiratory Enzymes
- Single circular DNA
- Flagella : for motility
- Pilli

EXCEPTIONS :

Some bacteria contain 2 chromosome : Vibrio, Leptospira Interrogans, Brucella

Lacks cell wall : MYCOPLASMA
Cell membrane has sterols : MYCOPLASMA
} Jumping jocker (Jocker of microbiology)

Lack muramic acid in cell wall : CHLAMYDIA

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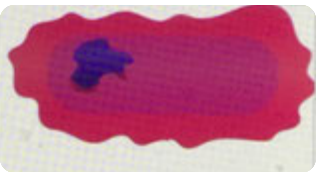
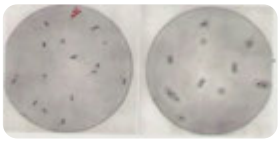
GLYCOCALYX

✓ 2 types : 1. Capsule
2. Slime layer



CAPSULE

- well defined layer
- Polysaccharide in nature
- Antiphagocytic
- Phage protection
- Most important virulence factor
- Repeated subculture leads to loose of capsule known as Smooth to Rough variation
- Capsule is antigenic
- On mixing the capsule with Anticapsular Ab leads to SWELLING OF CAPSULE
  ⇒ QUELLUNG REACTION

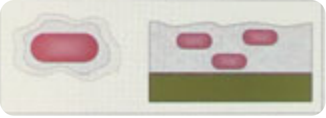


- Since it has NO NET CHARGE
  ↳ not stained by Gramstain
- demonstration can be done by NEGATIVE STAININ
  (India ink / Nigrosin)
- Capsulated bacterias :

Yersinia pestis	-Yes
S. Pneumoniae	-Some
V. Parahemolyticus	]Very
V. Cholera 0139	
Group A,B,C,D of β-strept	-Smart
Bacteroides fragilis (Zwitter ionic capsule)	]Bacteria
Bordetella pertussis (Non antigenic capsule)	
H. influenza	-have
Klebsiella	-killer
B. anthracis (Polypeptide Capsule)	- And
Menigococcus	- Mean
Clostridium perfringens	-Capsule

SLIME :

- loose ill demarcated layer around cell wall.
- is polysaccharide in nature
- When a group of bacteria growing together produces slime, the slime gets collected & form "BIOFILM"
  ↓
  * anti phagocytic
  * adhesion
  * mechanical barrier for antimicrobial entry



Eg : S. mutans
S. epidermis
P. aeruginosa

CELL MEMRANE

- it's a phospholipid bilayer
- Semipermeable in nature
- Enzymes responsible for uptake of nutrients known as PERMEASES are present
- All the cytochromes, resp. chain enzymes, synthetic enzymes of cell walls are present at the level of cell membrane.

SPORES

- produced under extreme conditions
- 2 genera under pathogenic bacteria that are spore forming Bacillus, Clostridium

Bacillus ↓	Clostridium ↓
- Non bulging spore	- Bulging spores
- only form in soil & culture	- form in soil, culture & in human body EXCEPT : C. perfringes - no spore in human

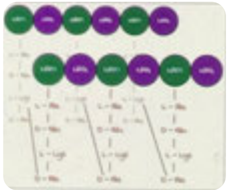
- STRUCTURE OF SPORES :

1. Core : contains genomes of bacteria
   Glycolytic enzyme
   Stored energy → as 3 phosphoglycerate
   Calcium dipicolinate (heat resistant)
2. Spore wall : surrounds the core
   typical peptidoglycan structure
   forms the future wall of vegetative bact.
3. Spore cortex : Next to spore wall
   Atypical peptidoglycan structure
   THICKEST LAYER
4. Spore coat : made up of Keratin like protein
   give Resistance to chemical disinfection
5. Exosporium : Lipoprotein in nature

CELL WALL

- made up of peptidoglycan cross-linked by peptides

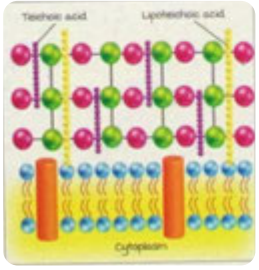
GRAM POSITIVE CELL WALL	GRAM NEGATIVE CELL WALL
1. ~ 80 nm thick	1. 10-25 nm thick
2. 50-100 layers of murein monomers cross-linked to each other by peptides	2. 2 layers of murein monomers cross linked to each other by peptides
3. Aromatic (or) Sulphur containing aa absent in cell wall	3. All types of aminoacids are present in cell wall
4. Contains Teichoic acid	4. Teichoic acid absent
5. Special structures Absent	5 Special structures like - Outer membrane - Periplasmic space - Endotoxin / LPS } present



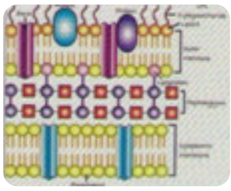
✓ In gram positive cell wall,

- murein monomer contains → N-Acetyl Glucosamine / → N-Acetyl muramic acid
- attached to every N-acetyl muramic acid, there is a Tetrapeptide which are cross-linked to each other by Pentapeptide cross bridges
- cross-linking maintains integrity of the cell wall.
- cross-linking is mediated by → Carboxy peptidases & Transpeptidase (Pencillin binding proteins / PBP)
  ↑ inactivated by β-lactam.

TEICHOIC ACIDS



✓ Role in adhesion & integrity of cell wall
✓ Cell wall teichoic acids : are Polymers of Ribitol phosphate
✓ Cell membrane teichoic acids (lipoteichoic acids)
↳ Polymers of GLYCEROL PHOSPHATE

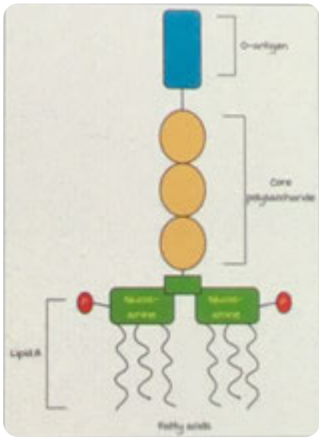


In gram -ve cell wall,

- The cell membrane beneath cell wall
  ↓
  It's a phospholipid bilayer
- Just above the cell membrane : Periplasmic space containing 2 layers of murein
- Outer to periplasmic space → Outer membrane phospholipid bilayer with
  | 2 proteins
  ↓ Integral / structural proteins
  ↓ Porins

- Embeded in the outer leaflet of outer membrane : Lipopolysaccharide / Endotoxin

LPS / Endotoxin



- 3 parts :

1) Lipid A : embeded in the outer membrane
actual endotoxic activity
inner most part

2) Core polysaccharide : short chain of 6-10 Carbohydrates
Unique carbohydrate present over here.
1. KDO – Ketodeoxy octanoic acid
2. Heptose

3) O/Somatic antigen : outermost part
Polysaccharide in nature
most variable part of LPS

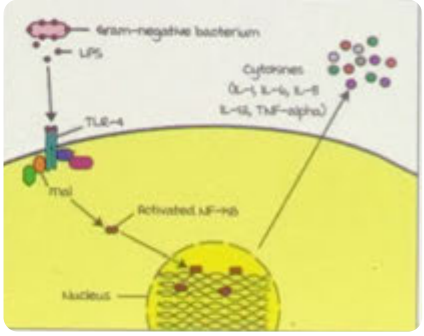
MOA : 1. Activates alternate complement pathway
2. Activates the tissue factor
3. Induces Cytokines release from innate immune cells on the surface macrophage & dendritic cells

↓

Toll like Receptors-4 (TLR-4) are present

↓

it recognise the LPS



Gram -ve bacterial infection releases LPS & binds to TLR-4

↓

Activate nuclear transcription factor (NF-KB)

↓

Releases cytokines (IL-1, 6, TNF-α)

Pharmacological effects of endotoxin in humans

- Fever - Most constant effect
- Fall in BP
- Increases the vascular permeability
- Increases the expression of adhesion molecule
- Intravascular coagulation → DIC ⇒ Shock

} Only seen with large amounts of endotoxin.

ENDOTOXIN	EXOTOXIN
- Lipopolysaccharide in nature	- Proteins / polypeptide in nature
- Present in the cell wall of G -ve bacteria	- Secreted by both Gram +ve & Gram -ve bacteria
- Released on lysis	- Actively secreted by the bacterium
- Heat stable	- Heat labile
- Poorly antigenic	- Highly antigenic
- Can't be toxoided	- Can be toxoided → used as vaccines
- Large amounts are required to see their pharmacological effect	- Minute amounts (ng/µg) can shows their pharmacological effects
- Constant effect	- Variable effects.

EXCEPTIONS:

1. Listeria (gram +ve) has LPS in cell wall
2. Botulinum toxin (exotoxin) → not secreted by bacterium, it's only released on the lysis of the bacterium

Limulus Amebocyte lysate assay (LAL Assay)

- detect nanograms of endotoxin in clinical specimen
- LIMULUS is a HORSESHOE CRAB, and it's bodycavity contain amebocyte.

↓

we extractout the amebocyte

↓

generate lysate

↓

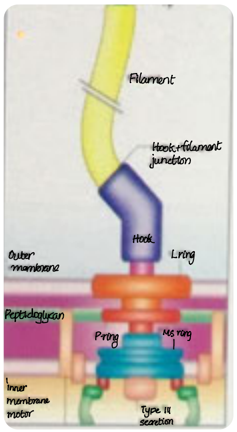
take a drop of it into a slide

if the clinical specimen contain a very small amount of endotoxin lysate shows Coagulation → Indicate presence of gram -ve bacteria

FLAGELLA

- made up of repeating subunits of the protein : Flagellin
  ↓
  ∴ antigenic in nature
- 3-20μ long & 0.01μ bread.
- Parts :
  - Filament
  - Hook
  - Basal body : made up of rings that is continously rotated by a proton dependendent pump.

    This rotation is transmitted to filament causing the motility.



- In gram -ve bacteria, basal body is made up of M, S, P, L
- In gram +ve bacteria, basal body is made up of M & S

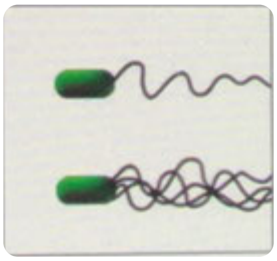
- Distribution of flagella

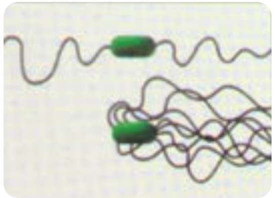
  Monotrichous : Single flagellum at one pole
  Eg : Vibrios, Pseudomonas aeruginosa

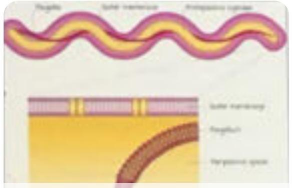
  Lophotrichous : multiple flagella at one pole
  Eg H. pylori, Campylobacter

  Amphitrichous : flagella on both the poles
  Eg : Campylobacter, Spirillum

  Peritrichous : Flagella all over the surface of the bacterium
  Eg : Bacillus, Clostridium
  Enterobacteriacea
  Vibrio paraheamolyticus (on solid media)
  Listeria (only at 25-28°C)







Endoflagellae :

- found in periplasmic space
- present in spirochetes

Demonstration of flagella

Direct method

a) Electron microscope
b) Leifson & Ryu impregnation stain

Indirect method

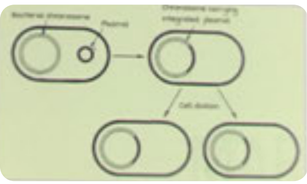
a) Hanging drop preparation
b) Soft agar medium
↓ (0.2-0.5%)
Eg: motility test agar
Cragie's tube
U-tube

Plasmid :

- Extra chromosomal double stranded DNA
- Circular
- In cytoplasm
- 1-40 plasmids may be present in a bacterium

- Not essential for life of bacterium
- Replication independant of chromosome

Plasmid integrated with chromosome known as EPISOME



- All plasmids transferred vertically
- Only some plasmid transffered horizontally via conjugation

That is the one with tra genes
↓
help in formation of sex pilus (conjugation)

Classification of plasmids

* Based on transfer

Conjugative

capable of both vertical & horizontal transfer
Eg: F/Sex plasmid
R plasmid
Col plasmid

Non-conjugative

Only vertical transfer

* based on their function mediated

1. F/Fertility plasmid → Conjugation
2. Resistance plasmid → Resistance to antibiotics
3. Col plasmids → Encode bacteriocin (antibiotic like proteins which only kill related Bacteria)

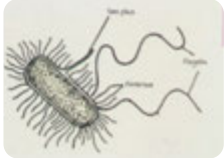
Types of motility

- corckscrew motility → T. pallidium
- darting/shootingstar → Vibrio + Campylobacter
- stately motility → Clostridium + salmonella
- end to end/tumbling → Listeria
- Gliding motility → Mycoplasma (noflagella)
- Twitching motility → Eikenall corrodens (noflagella)

Differential motility:

Non motile at 37°C &
Motile at 25-28°C
Eg: Listeria monocytogenes.
Yersinia enterocolitica
Yersinia pseudotubercolulosis

Pili / Fimbriae



- protein in nature made up of PILIN
- Types of pili

(i) Common pili
- mediate adhesion
- Only on gram -ve bacteria

(ii) Sex pili
- responsible for conjugation
- gram -ve & +ve bacteria
- bacteria producing the F Plasmid can produce sex pili

Examples of Bacteriocin

- E.Coli, shigella	: Colicins
P. aeroginosa	: Pyocins
Klebsiella	: Klebocins
C. diphtheriae	: Diphthericins

4. Metabolic plasmids:
Synthesise enzymes for metabolising special substrate Eg: Urea.

5. Virulence plasmids:
encodes special virulence factor
Eg: Capsule → B. anthrascis
Toxin → labile toxin of ETEC
→ Anthrax toxin

Gene Transfer

Primary mechanism of gene transfer

1. Transformation
2. Via bacteriophages
3. Conjugation

TRANSFORMATION

- uptake of soluble DNA fragments in the enviornment directly through cell wall.
- 1st demonstrated by : Griffith
  on : Strep. pneumonia
- DNA bacteria undergo lysis

↓

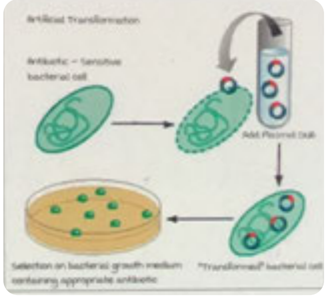
DNA fragments released into the enviornment

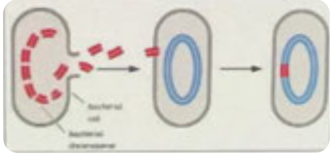
↓

Live bacteria takesup the DNA through cell wall

↓

Incorporate in to the genome

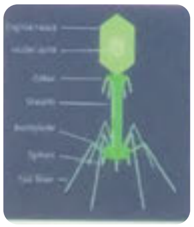

Artificial Transformation
Antibiotic – Sensitive bacterial cell
Add Plasmid DNA
Selection on bacterial growth medium containing appropriate antibiotic
"Transformed" bacterial cell



- Ability to take up DNA through the cell wall → Competence
- Artifiscial transformation → forced transformation → Use : Genetic Engineering

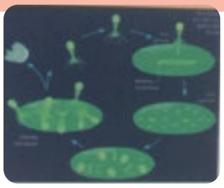
VIA BACTERIOPHAGES

✓ Bacteriophages : viruses that infect bacteria
✓ these are specific to certain bacterias
✓ they will bind to the receptors on bacterial cell wall



LYTIC CYCLE

- ingect phage DNA into bacterial CYTOPLASM



↓

Metabolic enzymes are used to synthesise phage component

↓

Assembly of daughter phages

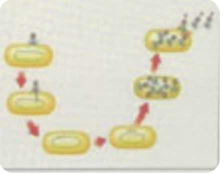
↓

Lysis of bacterium

↓

infect new bacterium.

LYSOGENIC CYCLE



Ingest phage DNA into bacterial cytoplasm

↓

Phage DNA get integrated in to bacterial chromosome

This phage DNA is called as : PROPHAGE

This bacterium is known as : LYSOGENIC BACTERIUM

↓

Exposed to uv / Nitrogen mustards
(or)
Sometimes even spontaneously

↓

Phage DNA disintegrates from the bacterial chromosome known as Induction of prophage

↓

enters the Lytic cycle.

✓ Phages which only can follow Lytic cycle : Virulent Phages
Eg: T1, T2, T3 Phage of E.Coli

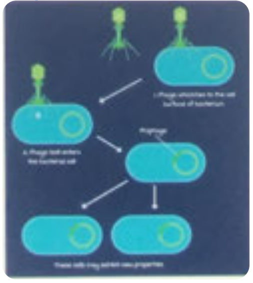
✓ Phages that can follow both cycle : Temperate phages
Eg: Lambda phage of E.Coli

✓ 2 mechanisms of gene transfer via phages
a) Lysogenic conversion
b) Transduction

LYSOGENIC CONVERSION

Prophage itself gives a new property to the Lysogenic bacterium which gets expressed

Eg:(i) Toxin production (Phage mediated toxins)

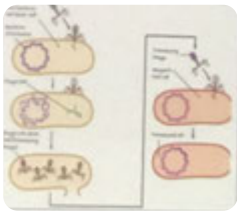
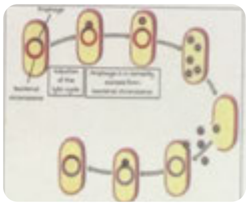


1. Diphtheria toxin (β phage)
2. Cholera toxin (CTX Φ phage)
3. Verocytotoxin (EHEC)
4. Panton Valentine leukocidin (S. aureus)
5. Pyrogenic toxins A & C (S. pyogenes)
6. Botulinum toxin C & D

Eg:(ii) Antigenic Variation
- Salmonella

TRANSDUCTION

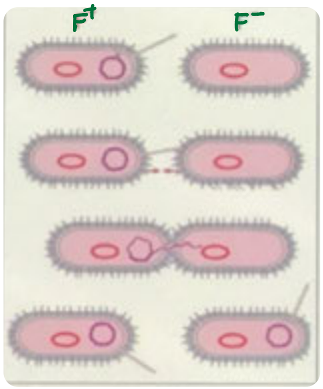
- Bacterial genes are transferred via a phage from one bacterium to another
- 2 types

GENERALISED TRANSDUCTION	SPECIALISED TRANSDUCTION
	
• occurs in lytic cycle • due to defective assembly (due to packaging error) • can be mediated by a virulent / temperate phage	• Occurs in Lysogenic cycle • due to defective excision during induction of prophage • only mediated by a temperate phage

VIA CONJUGATION

- via formation of sex pilus
- due to presence of F plasmid / sex plasmid / Fertility plasmid (also known F Factor / Transfer factor)
- F^+ bacterium : F plasmid is present
  F^- bacterium : F plasmid is absent.

A. Simple conjugation

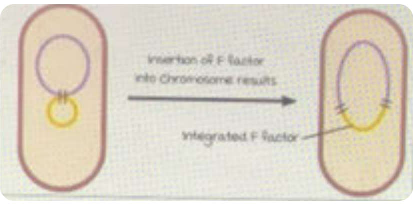


- first demonstrated by Lederberg & Tatum on E Coli K12 strain

F^+ bacterium + F^- bacterium
↓
Sex pilus formed by F^+ like a bridge to F^-
↓
One strand of F plasmid get transffered to F^- bacterium
↓
Both bacterium produce complimentary strand to the single strand (template strand) in them
↓
Both the bacterium thereby, gets the ability to form sex pilus

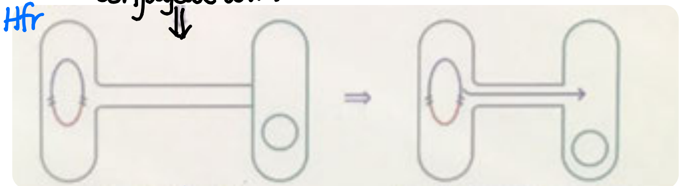
B. High freequency Recombinant Conjugation (HFR)

- F plasmid is integrated with the chromosome (Episome) in this bacterium



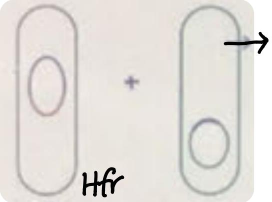
Hfr

Conjugate with F^-
⇓



Sex pilus formation

The chromosomal genes are the initial ones to get transffered
⇓
Before the complete strands get transffered either the sex pilus / chromosome itself breaks
⇐
only some chromosomal genes are getting transffered (not the F plasmid genes] to F^- bacteria
⇓
Recombinant F^- formation

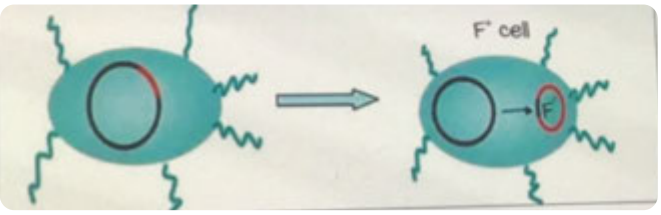


→ Recombinant F^-

∴ Equation
$Hfr + F^- \Rightarrow Hfr + Recomb.\ F^-$

ie the Hfr state is reversible

C HFR is reversible



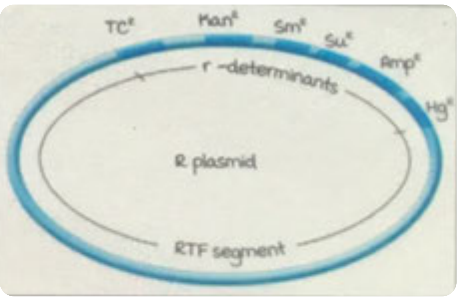
When the F plasmid is disintegrating from the bacterial chromosome, it takes away some chromosomal genes next to the site of integration and form F' plasmid (F prime plasmid)

F plasmid (Independent of chromosome)
Chromosomal genes (next to site of integration)
} ⇒ F' plasmid

i.e $F' + F^- \Rightarrow 2F'$

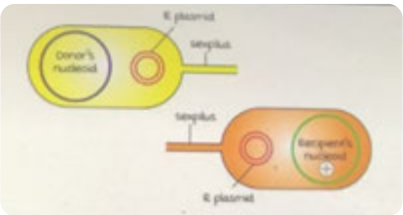
THE TRANSFER OF F' ⇒ SEXDUCTION

D. R Plasmid / R factor



F Plasmid + Antibiotic resistance genes (r determinant)
⇓
now this F plasmid is known as Resistance transfer factor

- Transfer of R plasmid via conjugation
⇓
MC mechanism of spread of antibiotic resistance.



- first demonstrated on Shigella

E. Col plasmid

→ F plasmid + Bacteriocin genes (Antibiotic like proteins that kill related bacteria)

NOTE:

- The nature of transforming principle is DNA was demonstrated by Avery et al & co.
- bacteriophage was first described by : F.W Twort
  name coined by : De' Herelle

Telegram - @Datesbugnotes

Staphylococcus

✓ Family : Micrococcasea → contains Staphylococcus & Micrococcus

	STAPHYLOCOCCUS	MICROCOCCUS
Arrangement	Clusters	Tetrads
O_2 requirement	Facultative anaerobes	Strict aerobes
Glucose utilisation	Fermentative	Oxidative
Catalase	+	+
Oxidase	−	+
Bacitracin	Resistant	Sensitive
Lysostaphin	Sensitive	Resistant

STAPHYLOCOCCUS

- Gram +ve
- Arranged in clustures
- Non motile, non sporing
- Facultative Anaerobes
- Microcapsule +/−
- All species are Resistant to 7-10% salt

Biochemistry:
Catalase +ve
Oxidase −ve
Ferment sugars
Nutritionally nonfastidious

Classification:

On the basis of coagulase enzyme

Slide coagulase test → Detection of bound coagulase
Tube coagulase test → Detection of free coagulase

↓ if both are +ve
↓ Staph aureus
↓ Produces Golden yellow pigment
β-hemolytic
mannitol fermentation +ve

↓ one/both are -ve
↓ all other staphylococcus
↓ CONS : Coagulase negative Staphylococci

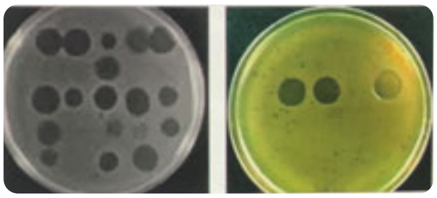
Eg: S. suprophyticus } NOVOBIOCIN
S. xylosus } RESISTENT
S. ludgenesis } BOUND
S. schleifri schleifri } COAGULASE
S. intermedium } FREE
S. schleifri coagulans } COAGULASE

Staph. Aureus

TYPING: (a) Serotyping
(b) Biotyping
(c) Phage typing
↳ 23 standard bacteriophages are used
↓
there are 5 phage groups/types

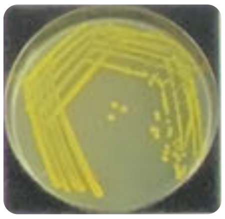
- Phage type III : most commonly associated in MRSA strain
- Phage typing center for S. aureus → Maulana azad medical college.

Take a nutrient agar plate → Swab inoculate specimen over the medium
↓
at specified site drop tiny amounts of 23 bacteriophages
↓
after overnight incubation special lytic pattens are formed



Cultural Charachteristic of Staph. aureus

On Nutrient Agar → Produces Golden Yellow pigment
Non diffusible pigment
↓
"STAPHYLOXANTHINE"



it's production is increased by
(i) incubation at 22°C
(ii) Aerobic condition
(iii) add Tween 80/milk/glycerol monoacetate

On Blood agar → pin head colonies
β-hemolytic

On MacConkey agar → pink coloured colonies
Lactose fermenters

Biochemical reaction

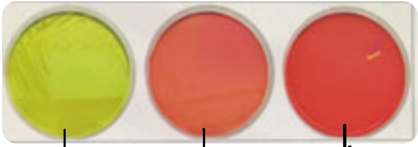
- Slide, tube coagulase +ve
- mannitol fermenting
- urease +ve
- DNAase +ve
- Thermonuclease +ve
Phosphatase +ve
- Nitrate reduction +ve

Selective media for Staph. aureus

⇒ Salt agar
⇒ Salt milk agar
⇒ LUDLAM medium

Selective + Differential medium

Mannitol Salt agar



Infections :

- MC infection : Skin & Soft tissue
  Eg: Stye
  Folliculitis
  Carbuncle
  Furuncle
  Bullous impetigo
  Mastitis
  Botiomycosis
  Post-operative wound infection
  } MOST COMMON CAUSE : Staph. aureus
  Other infections : Impetigo
  Lymphangitis
  Cellulitis
- Bone, muscles & joints :
  - Osteomyelitis
  - Septic arthritis (children & adults)
  - Septic bursitis
  - Myositis/Pyomyositis
  } MC Cause: Staph. aureus

Normal Carriage sites :

- MC site : Nose (30-50%)
  t/t: Ointment MUPIROSIN
  thrice a day × 5-7 days.
- Oropharynx
- skin
- hair
- Perineum
- Vagina

Toxin mediated diseases
- Toxic shock syndrome
- Scalded skin syndrome
- Food poisoning

- Respiratory tract infections : all infections
  - ↳ MCC of lung cysts / Pneumatocele
- MCC of Native Valve Endocarditis
- single MCC of Early & intermediate Prosthetic valve endocarditis
- MCC of infective endocarditis in IV drug users
- it can also cause : Bactremia, Meningitis, UTI

Pathogenicity factor

1. Cell wall associated

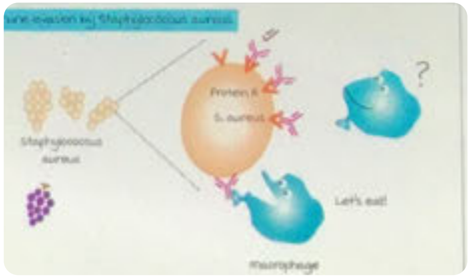
(i) Slime +/−

(ii) Microcapsule +/− ⇒ ZWITTER IONIC (like Bacteroides fragilis)

(iii) Adhesion proteins ⇒ Collagen binding
Fibronectin binding

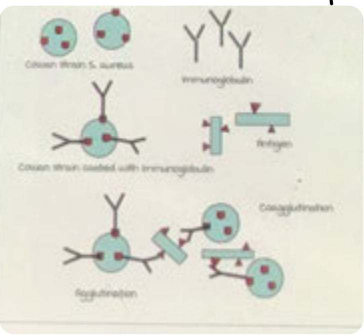
(iv) PROTIEN-A ⇒ Binds to Fc part of IgG → and make it Antiphagocytic

macrophage receptors on Fc of IgG is masked by protein A



USES :-

→ COAGGLUTINATION : to detect soluble antigen



Staph. aureus with Protein A
↓
mix it with Ab of Protein A
↓
mix it with clinical specimen containg soluble Ag → Ag in clinical specimen binds to Abs over Staph. aureus crosslinking occurs → visible agglutination

- usually soluble Ag shows precipitation. But here we convert that to Agglutination test.
- 'COWAN I' strain of S. aureus is used (rich in protein A) in coagglutination

(v) BOUND COAGULASE
- it activates prothrombin

Fibrinogen $\xrightarrow{\text{prothrombin}}$ Fibrin act as a ANTIPHAGOCYTIC barrier for S. aureus

- it's of only 1 antigenic type
- Detection : Slide coagulase test
- Absent in about 12% cases of Staphylococcus.

Virulence factors secreted by Staphylococcus

(i) Free coagulase
- MOA : similar to that of Bound coagulase
- 8 antigenic subtypes
- it requires Coagulase Reacting factor for optimal action (absent in Guineal pig plasma)
  So, in tube coagulation test → donot use Guinea pig plasma
- Present in 100% strains of Staph. aureus

(ii) Hemolysins ; α, β, γ, δ

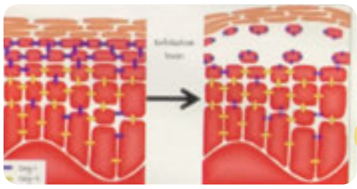
α-hemolysin	β-hemolysin	γ-hemolysin
• Most important • it's L - Lethal D - Dermonecrotic L - Leucocidal • it shows PARADOXICAL REACTIVATION : if we start to heat α hemolysin it gets inactivated at 60°C & reactivated 80-100°C	• Sphingomyelinase enzyme. • it shows HOT-COLD Phenomenon hemolysis of RBC begins at 37°C completed at 4°C • α-toxin of C. perfringens also shows the same	• bicomponent toxin • it has membrane damaging property • As both of this are present called as SYNERGO-HYMENOTROPIC toxin

(iii) Panton valentine Leucocidin :

- phage mediated toxin (Lysogenic convertion)
- synergo-hymenotropic toxin
- dermonecrotic & Leucocidal proparty
- secreted only by 2-5 % strains of Staphylococcus
- BUT almost 100% of community acquired MRSA secretes this toxin

(iv) EPIDERMOLYTIC TOXIN/EXFOLIATIN

- 2 antigenic types
  EF - A → Chromosomal mediated
  EF - B → Plasmid mediated
- Proteolyse DESMOGLEIN-1
  ↓
  present in desmosomes
- Site of action : Stratum granulosum
- But the receptors for this toxins present in
  - Neonates
  - Young adults
  - Adults with skin diseases.



- Cleavage of desmosomes
  ↓ leads to
  MID-EPIDERMAL SPLIT
  ↓
  Generalised Exfoliation
  ↓
  Charachteristic feature of RITTER's DISEASE (or) Scalded Skin Syndrome

Diagnosis : ELISA on the serum for EF-A/B

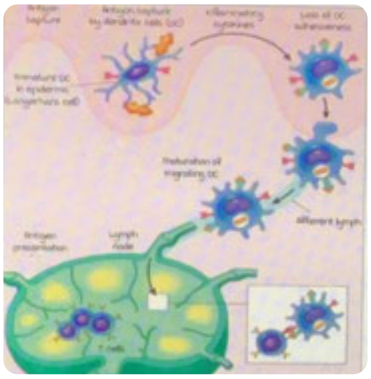
Treatment : Topical care, Parenteral anti-staph antibiotics

(v) ENTEROTOXINS

- secretes 15 antigenic types SE A, B, C....
- highly heat stable → 100°C for 10-40 min
- MOA : Stimulation of Vagus & Vomitting center
- Cooked food/milk products/potato salad contamination by carrier
  ↓
  has pre-formed toxins
- Staph : food poisoning
  ↓
  - I.P = <6 hrs
  - patient with Nausea, Vomiting watery diarrhea
  - spontaneous resolution → 10-12 hrs
  - Diagnosis : ELISA → Stool
    Vomitus
    Food
  - Treatment : NO ATIBIOTICS
    Symptomatic t/t

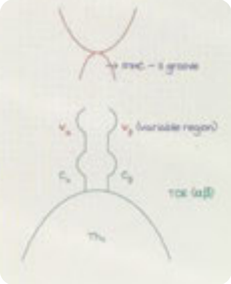
(vi) Toxic shock Syndrome toxin -1 (TSST -1)

- Earlier called as Enterotoxin F
- PYROGENIC TOXINS SUPER ANTIGEN



Whenever Ag enters the body,
if innate immunity unable to control
↓
antigenpresenting cells comes in to action
↓
Most specific Ag presenting cell - Dendritic Cell
↓
Dendritic cells takes up the Ag & phagocytose it
↓
Processed in Lysosomes
↓
Genenerate small-small peptides

it conjugate peptide into pathogen ← in the grove of MHC-2
↓
migrate to regional lymphnode
↓
wait for helper T Cell to recognise peptides of pathogen by Anigen specific Receptors → During peripheral recirculation come & regonise peptide
↑
Leads to adaptive immunity



After recognition → Immunological synapse with dendritic cell
↓
Secrete specific CYTOKINES & goes to either TH_1, TH_2 (or) TH_{17}
↓
Depending which cytokine & pathogen are taken up by Dendritic cell.
↓
finally CELL MEDIATED IMMUNITY will act.

ADAPTIVE IMMUNE RESPONSE

1st basic principle :

- Each T & B - cell meant to recognize different Ag.
- Every T & B cells has their own Antigenic Specific Receptors
- This is called 'Receptor Diversity'

2nd basic principle :

- All T & B cells undergo constant peripheral re-circulation, looking for Ag.
- It means 2-3 days every T & B cell will enter every follicle of MALT, SPLEEN, LYMPH NODE
- known as CONSTANT PERIPHERAL CIRCULATION

3rd basic principle :

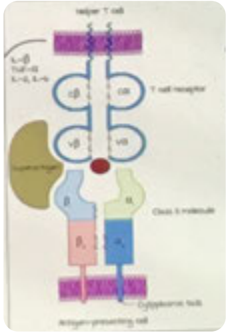
- On recognizing particular Antigen
↓
That particular T or B cell is selected out to undergo expansion
↓
Results in formation of
- Effector T & B Cell
- Memory T & B Cell
↓
So CLONAL SELECTION & EXPANSION
Selected undergo proliferation

In normal circumstances :

Normal Ag → < 0.0001% T_H are activated → Expands & produce cytokine
↓
Leading to adaptive immunity

SUPER ANTIGENS

- Donot need processing by APC
- Also called as TCELL MITOGEN
- Directly binds to MHC-II at the site lateral to usual antigen presenting groove.
- Need recognition by just V_β part of TCR
- 5-10% of T_H cells sharing same V_β part of PCR



↓
Activated → Expansion → Massive Cytokine release
↓
Responsible for Toxic Shock Syndrome

Causes of Staphylococcal TSS
- TSST-1
- Staphylococcal Enterotoxin B
  Enterotoxin C

CDC CRITERIA FOR TSS

- CDC : Center of disease control

1) Fever (>102°F)
2) Reduced BP (Systolic BP <90mmHg)
3) Generalised Maculopapular rash
4) Desquamation of maculopapular rash in 10-14 days
5) 3 or more organ system involvement

GIT	→	Nausea, Vomiting
Mucosa	→	Hyperemia
Kidney	→	Creatinine (2 times)
Liver	→	SGOT/SGPT (2 times)
Muscle	→	CPK (2 times)
Platelet	→	< 1 lack/mm^3
CNS	→	mental confusion

6) Negative result of
   - Blood
   - Throat
   - CSF

   } Cultures for other bacteria should be -ve
   - Negative serology of Ricketsia
     Leptospira
     Measles

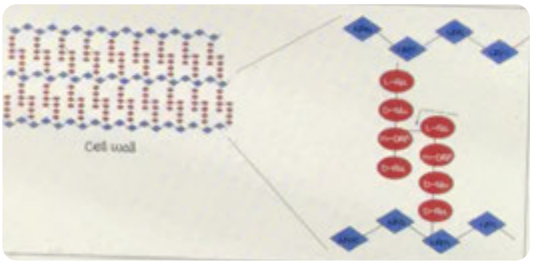
TYPES OF TSS

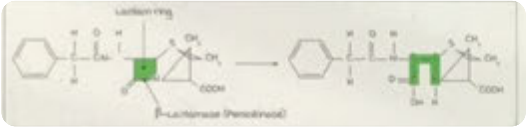
1. **Menstrual TSS**
   - use of Tampons during menustruation along with colonisation of S.aureus
   - Use of tampoons → ↑ TSS toxin production
   - 90-100% cases Menustrual TSS → are due to TSST-1
2. **Non-menustrual TSS**
   - MCC → Staph. Enterotoxin B
     Staph. Enterotoxin C
   - In untreated cases → 5% mortality
     But in Strep. TSS → 50% mortality

DIAGNOSIS & TREATMENT

- ELISA on serum
- Symptomatic t/t
- Antibiotics
- CLINDAMYCIN + CLOXACILLIN
  (or)
  Vancomycin (in MRSA)

BETA-LACTAM RESISTANCE





- β-lactam → binds to transpeptidases / transpeptidases
  ↓
  And prevent the cross-linking
- MC mode of Resistance → Ability to secrete β-lactomases
  it hydrolyses ring
- β-lactams are encoded in R plasmid
  MC spread of R plasmid → Transduction

MRSA/ORSA :-

- methicillin resistant / Oxacillin resistant
- There is a mobile genetic element present on chromosome known as SCC mec (Staphylo coccal Chromosomal Casette mec).

among them
↓
mec A gene
↓ encodes
Altered Transpeptidase : PBP2a or 2'
∴ β-lactam can't bind

Diagnosis of MRSA :

1. Dilution method → if Oxacillin MIC ≥ 4µg/ml
2. Oxacillin Agar screen → it contain 6 µg/ml Oxacillin. if any growth is there
3. Disc Diffusion → Cefoxitin (superior) or Oxacillin discs are used
   ↓
   mec A gene is better expressed in Cefoxitin than oxacillin
   - mec A gene is better expressed in lower temp. ∴ incubate Antibiotic plate at 30-33°C
4. Latex Agglutination (Rapid test) → for altered PBP's
5. Gold standard → PCR for mec A

Treatment of MRSA :

1) Put in a private room (or in room with other another MRSA patient)
2) Hand hygeine : disinfect hand with Chlorhexidine hand wash after examination
   ↳ MC mode of spread in hospitals (hands of health care women)
3) Wearing of PPE when entering the room (gowns & gloves)
4) Only transporting out of room for essential test
5) Antibiotics :

Vancomycin
Daptomycin
Linezolid
Fluroquinolones

Streptogramins
Septrans
β lactams → 5th generation cephalosporins (Ceftobiprole, Ceftroline)
→ Carbopenenams (Razupenam)

TYPES OF MRSA

COMMUNITY ACQUIRED MRSA	HOSPITAL ACQUIRED MRSA
• if there is No such history in last 1 year	• Current or recent exposure to - Health care worker - Hospital, rehabilitation, dialysis center - Indwelling venous catheters
• SCC mec - IV, V, VI types on gene sequencing	• SCC mec I, II, III on gene sequencing
• more virulent strain	• less virulent
• ~ 100% produce PVL	• No such association
• Relatively sensitive to othe antibiotics	• often multidrug resistant

VRSA : (few reports)

- 'van a' gene on plasmid / transposon acquired from VRE - Vanco resistant Entero coccus
- 'van a' encodes : Altered cell wall precurssors
- often MRSA
- by dilution method → MIC of vancomycin is ≥16 µg/m
- Teicoplanin → also has high level of resistance

S. Epidermidis

- CONS (Coagulase Negative Staphylococci)
  - mannitol fermentation: -ve
  - Novobiocin sensitive
  - Non hemolytic
  - Non pigmented
- Normal skin flora
- Virulence factor → produce slime
- MCC of Bacteriamas in IV catherised
  Orthopedic implants
  Dialysis associated peritonitis
  Post lumbar puncture Meningitis
- Also causes :
  - Post op. wound infections
  - Native valve endocarditis
  - Prosthetic valve endocarditis
- Inherently Multidrug resistant
- DOC → Vancomycin

S. saprophyticus

- Novobiocin resistant
- Coagulase negative staphylococci
- mannitol -ve
- Non hemolytic
- Non pigmented.
- Normal skin flora & Female Genito urinary tract flora
- Most common infection :
  - After E.Coli, MCC of UTI in young females

beta haemolytic streptococcus

- ✓ family : Streptococcacea
- ✓ Gram +ve cocci, seen as chains/pairs
- ✓ Non-motile, non-sporing
- ✓ Fac anaerobes
- ✓ Group A, B, C, D of β-hemolytic → Capsule present

Biochemical reactions

- ✓ Catalase test -ve
- ✓ Oxidase test -ve
- ✓ Ferment sugars

Cultural characteristic

- ✓ Nutritionally fastidious
- ✓ Only grow on enriched media

Classification

- Brown classification : based on hemolysis on blood-agar
  Pour-plate culture

β-hemolytic

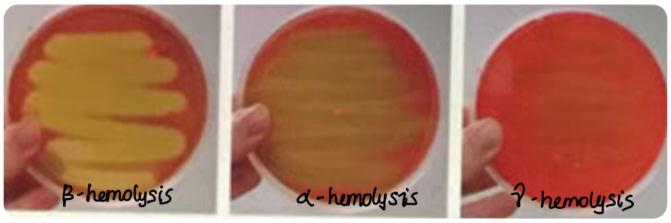
- Complete hemolysis
- Yellow colonies

α-hemolytic

- Green colonies

γ-hemolytic

- No hemolysis



BETA-HEMOLYTIC STREPTOCOCCI

- ✓ further serogrouped by → Lancefield
  on the basis of C carbohydrate Ag present
  on cell wall
- ✓ Group A to V, except I & J
- ✓ Most pathogenic : Group A & Group B

Methods of extraction of C carbohydrate Ag:

- Lancefield method → HCl used
- Ranz & Randall → Autoclaving
- Elkholy's method → Nitrous acid
- Maxted method → Pronase
- Fuller's method → Formamide

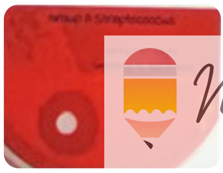
Original Lancefield serogrouping done by : Ring precipitation test

GROUP A / Strept. Pyogenes

- ✓ Most pathogenic
- ✓ Capsulated (only 1 capsular serotype) ∴ Typing can't be don the basis of capsule.
- ✓ GRIFFITH TYPING : Based on their M proteins in Cell wall
  - >120 M types
  - Subtyping on basis of T & R proteins
  - Earlier M types like M1, M3, M6 ⇒ Respiratory infection
    Later M types like M49, M57, M61 ⇒ Skin & Soft tissue infection. mainly

Biochemical Reactions

1. MASTED'S OBSERVATION → Sensitive to Bacitracin
2. Not fermenting Ribose (most strept. ferment Ribose)
3. PYR test +ve (Pyrolidonyl amidase)



Culture Charachteristics :

- Blood Agar :- Pin point colonies, β-hemolytic
  - MUCOID / MATT APPEARANCE : → Virulent strain ie Capsule + & M proteins +
  - GLOSSY APPEARANCE → Avirulent strain ie Capsule -; M protein -
- Transport medium : Pike's medium

  Selective medium : Crystal violet blood agar

Virulence factors

A. Cell wall associated :

(i) Hyaluronic acid capsule (antiphagocytic)

(ii) Adhesion proteins (Eg: Collagen binding protein)

(iii) Teichoic acids

(iv) M Proteins :

- most important virulence factors
- COO^- terminal embeded in cell membrane
- amino terminal on surface
- Anti phagocytic & anti-complementary
- Type specific protective antibodies
- Encoded by 'emm' gene

B. Secreted virulence factors :

1. Hemolysin / Streptolysin (SL)

Streptolysin - O	Streptolysin - S
• O_2 labile	• O_2 stable
• Active in reduced state	• Active in oxidised / reduced state
• Hemolysis only in pour-plate cultures (i.e when you are making the colonies formed inside medium)	• Hemolysis around surface colonies
• It's L - Lethal C - Cardio toxic L - Leucocidal	
• it's Antigenic & form ASO Ab's ⇒ used in Diagnosis of Rheumatic fever	• Non antigenic → ∴ no Ab
• Also produced by Grp C & G strept.	

2. Streptodornase / DNase :

- depolymerises DNA
- Responsible for serous charachters of EXUDATES
- 4 types : A, B, C, D

  B → Most pathogenic
- Anti-DNase B titer → D_x of PSGN
- Use : Liquefaction of thick pus cells

3. Streptokinase / Fibrinolysin :

- Activates Plasminogen
- Responsible for spread of Strep. infection along with hyaluronidase
- Also produced by Grp. C & K strept.
- S. equisimilis of Group C produces Streptokinase & it's use

  Use : Thrombolysis

4. Dick/Pyrogenic/Erythrogenic/Scarlatini form toxin:
- it's a super antigen
- A & C : Phage mediated
  B : Chromosomally mediated
- Causes : Streptococcal toxic shock syndrome
  Scarlet fever

5. Hyaluronidase/Spreading factor
6. NADase / DPNase
7. Serum opacity factor

Infections caused by S. pyogenes

✓ MC Infection : Pharyngitis (sore throat)
✓ MC cause of bacterial sore throat
✓ Further complicated by otitis media, sinusitis, mastoiditis, Quinsy, Ludwigs angina

Skin & soft tissue Infection (SSTI)

- S. pyogenes is the commonest cause of :
  N - Necrotising fascitis / flesh eaters disease
  I - Impetigo
  C - Cellulitis
  E - ERYSIPELAS
  L - Lymphangitis
- Also cause : - puerperal sepsis
  - Endocarditis
  - Septic arthritis

Toxin mediated disease :
1. Toxic shock syndrome → 50% mortality
2. Scarlet fever

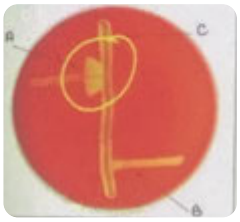
Non-suppurative complication

- Due to immunecomplex deposition
  1. Rheumatic Fever : following sore throat
  2. Glomerulo nephritis : following sore throat (or) SST (
     ↳ Caused only by nephritogenic M Types : M1, M3, M49, M57 ....

Treatmen : DOC : β-lactam (no resistance)

Group - B Streptococcus

- S. agalactiae : Capsulate, 10 capsular antigen
- Identification
  1 CAMP test +ve :



On blood agar plate → Inoculate streak of S. aureus
↓
Overnight incubation ← Streak S. agalactiae at right angle
↓
Enhanced zones of β-hemolysis (Arrow-head hemolysis)
due to synergism b/w β hemolysis of S. aureus & the CAMP factor, which is the hemolysin of S. agalactiae

2. Hippurate hydrolysis

Culture : Blood agar : pin-point, β-hemolytic
Islam's or Granada's medium : Orange coloured pigment

- S. aglactiae is a normal GI flora & female genito-urinary tract flora

Infections :

1. Neonatal disease :
   a. Early onset : with 1st 6 days of life
      - Perinatal transmission
      - caused by I, III, V capsular subtypes
      - manifest as : Septicemia, meningitis, Pneumonia
   b. Late onset :
      - Present b/w 7th day - 3 months of life
      - most commonly acquired, ocassionaly perinatally
      - capsular serotype III is major cause.
      - manifest as : Septicemia, Meningitis

Prophylaxis of perinatal transmission :

- CDC guidlines : at 35-37 weeks → Rectal & lower vaginal swabs taken ↓ if culture positive → Intrapartum pencillin prophylaxis

2. For other age group -
   MC cause of : Puerperal sepsis
   Pneumonia
   Endocarditis

DOC : β-lactams

Group-C Streptococcus

includes : S. equi
S. equisimilis → Source of streptokinase for Thrombolysis

- Animal pathogens
- In humans → cause : Respiratory tract infection (Pneumonia)
  endocarditis

alpha AND gamma haemolytic streptococcus

VIRIDANS GROUPS V/S S. PNEUMONIAE

VIRIDANS GROUP	STREPTOCOCCUS PNEUMONIAE
Chains	Pairs
Convex	Carrom coin
Bile insoluble	Bile soluble → Best
Optochin Ⓡ	Optochin Ⓢ
Inulin fermentation -ve	Inulin fermentation +ve

VIRIDANS GROUP ⇒ oral & upper respiratory flora

(i) S. mitis
(ii) S. mutans
(iii) S. sanguis
(iv) S. salivarius
(v) S. anginosus

↳ Non PATHOGENIC & HAVE LONGEST CHAINS

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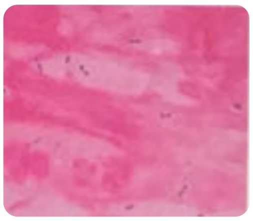
MCC of Late prosthetic valve endocarditis → MC → S.sanguis
MCC of Sub acute bacterial endocarditis → MC → S.sanguis
MCC of Dental carries → S. mutans

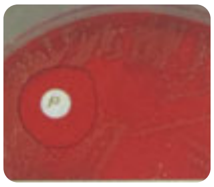
S. pneumoniae



- Gram positive ; diplococci
- Lancet shaped
- Capnophilic (5% CO_2) → enhance it's growth
- Capsulated organism

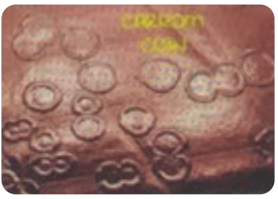
IDENTIFICATION :-

(i) OPTOCHIN SENSITIVITY
(ii) Bile solubility Test :
Bile salts ↑ the activity of **Autolytic enzymes** (Amidases)



Cultural Charachteristics :-

(i) On blood Agar : α-hemolytic colonies
(ii) In anaerobic conditions
↓
β-hemolytic colonies
(Because of O_2 labile hemolysin)
(iii) Carrom coin appearance
(central depression & peripheray raised)
also called as DRAGHTSMAN Appearance



Treatment :

β-lactam - Reports of resistance - **Altered PBP**

Vaccines [Subunit]
(a) Polysaccharide [Pneumovax] **23 capsular Ag**
- single dose I.M → 5 years of protection
Also recommended for patients
  - HIV +ve
  - Steroids recieving patients
  - Chronic hemolytic anemia
  - Chronic lung, liver, heart, Kidney disease
  - Age >65 yrs
  - Splectomised

(b) Conjugative vaccine - PREVNAR-13
- for - <2 years
- 13 capsular antigens conjugated to carrier protein
CRM 197 → Non toxic toxin of mutant C. diphtheriae

Typing based on capsule : >90 types :-

Serotypes based on "QUELLUNG REACTION"
Swelling of capsule in presecence of specific anticapsular anti-serum

Serotypes :
1 to 8 ⇒ >75% Adult infection
6, 14, 19, 23 ⇒ >75% Children infection
3 ⇒ most virulent (thick capsule)

Virulence factors :-

(i) Polysaccharide capsule : Anti-phagocytic
(ii) Adhesion proteins
(iii) Oxygen labile pneumolysin
(iv) IgA_1 protease

Infections by S. pneumoniae :-

• Normal Upper respiratory tract → flora → 20-50% individuals

Non Invasive infections (NO BACTREMIA)
(i) MCC of Otitis media
(ii) MCC of Sinusitis
(iii) After H. influenza ; MCC of excerebation of COPD

Invasive infections
(i) Pneumonia :
MCC of Community Acquired Pneumonia
(ii) Meningitis
MCC invasive in all except :
Neonates
HIV
15-25 yrs
(iii) Osteomyelitis
(iv) Septic arthritis
(v) Arthritis

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Non hemolytic / γ hemolytic streptococci

- It's GROUP D Streptococci

	ENTEROCOCCUS	NON-ENTEROCOCCAL GROUP-D
Hemolysis	γ : ocassionaly α/β	Only γ
Bile resistance	Resistant	Resistant
Aesculin hydrolysis	+ve	+ve
Growth in 6.5% NaCl	+ve	-ve
PYR test	+ve	-ve

→ Bile resistance & Aesculin hydrolysis done in same medium together called as Bile aesculin +ve

→ Bile resistance test : Resistance to 40% of bile

NON ENTROCOCCAL GROUP-D

- S. bovis (now renamed to)
  - → S. gallolyticus → Septicemia & endocarditis in colorectal cancer & Rectal polyps
  - → S. infantarium
- S. equinus

∴ sometimes present in normal GI flora in humans & animals

ENTEROCOCCUS

✓ Gram positive cocci
✓ Pairs/short chains
✓ Bile aesculin test +ve
✓ 6.5% NaCl +ve
✓ PYR +ve
✓ Grow at temperature → 10°C → 45°C
✓ Survive 60°C for 30min, grow at pH : 9.6
✓ Resistance to Sodium azide

CULTIVATION :

- In blood agar : γ hemolytic
  ocassionally → α, β hemolytic
- In MacConkey Agar :
  Tiny lactose fermenting colonies
- Selective medium : Sodium Azide agar

INFECTIONS :-

- Normal flora in : Oral
  GIT
  Genitourinary tract
- In infections : UTI
  Intra abdobinal abcesses
  Peritonitis
  Endocarditis
- 1st MC infectious agent : E. faecalis (80-90%)
  2nd MC infectious agent : E. faecium

TREATMENT :

- Inherent resistance to Pencillins
- Inherent low level Ⓡ to Aminoglycosides
- Synergism b/w Pencillins & AMGs
  ↓
  overcome this resistance
- Usual t/t : Ampicillin + Gentamicin
- If high level of Ⓡ to AG's
  DOC → Vancomycin

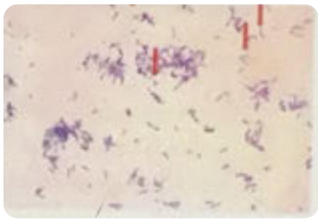
Vancomycin Resistant Enterococcus

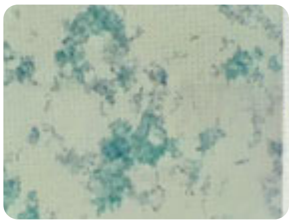
- Usually Vancomycin → Act on terminal cell wall precurssors : D-ala-D-ala
- it start to synthesize Altered cell wall precurssors
- it's due to VAN A gene, VAN B gene } D-ala-D-lactate
  VAN C gene
- Vanco Ⓡ >>> in E. faecium than E. faecalis

Corynebacterium

- Coryne → means 'Club' shaped swelling on either 1 or both ends
- Diphtheroids : Normal flora in body
  - ↳ Oral cavity : C. hoffmani
  - Conjuctiva : C. xerosis
  - Axillae, groin : C. jeikium, C. urealyticum
    - ↳ Bacteremia in Immunocompromised
- Lipophilic species : C. jeikium, C. urealyticum, C. kroppenstedtii, C. bovis

C. DIPHTHERIAE





✓ Pathogenic & most important species
✓ Gram positive
✓ Non motile, Non sporing, Non capsulated
✓ Aerobes & Facultative anaerobes

- Due to incomplete seperation after binary fission → Chinese letter arrangement / cuneiform arrangement
- Metachromatic granules / Volutin / Babes ernst / Polar Bodies
  - ↳ These are cytoplasmic granules composed of Poly metaphosphates :
    Acts as Energy stores for the bacteria.
  - Special stains to demonstrate these granules are : Albert (MC used)
    Neisser, Ponder's stain
  - In Albert stain : CYTOPLASM : GREEN (more Gram +ve than bacterial cytoplasm)
    GRANULES : PURPLE - BLUE
  - Albert stain = Malachite green + Toluidine Blue + Iodine

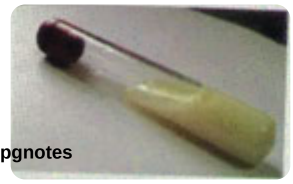
Cultivation

✓ fastidious organism
✓ Enriched medium : Loefflers serum slope
  Growth with in 4-8 hrs of incubation ←
✓ Selective medium : Mediums containing K^+ Tellurite in conc of 0.3 - 0.4 %
  - Macleod
  - Downie
  - Tinsdale / Cystine Tellurite agar
  - Hoyle
  - → Pottassium tellurite converted to Metallic tellurium

Biochemical Reactions :

Catalase +ve
Oxidase -ve
Ferment sugurs (anaerogenic) → no CO_2, only acid
Hiss serum sugur solutions
Nitrate reductase +ve
Urease test -ve

- Urease test used to differentiate C. ulcerans & C. pseudotuberculosis



Black shiny colonies on K^+ tellurite medium

Biotypes:	GRAVIS	INTERMEDIUS	MITIS
(i) Morphology	Short roads ʒ few granules	Long rods ʒ few granules	Long roads ʒ abundant granules
(ii) Colony	DAISY HEAD	FROGS EGG	POACHED EGG
(iii) Hemolysis	Variable	Non-hemolytic	Hemolytic
(iv) Startch & Glycogen fermentation	+	–	–
(v) Toxigenic strains	~100%	95-99%	80-85%
(vi) Complication (MC)	Paralytic & Heamorrhagic	Heamorrhagic	Obstructive complication

(i), (ii), (iii), (iv) are criterias for biotyping. But (v) & (vi) are additional criteria

- Another biotype : "Belfanti" → Nitrate reduction test –ve strain
- Phage typing : 15 bacteriophage types
- Genotype typing: Ribotyping, pulsed field gel electrophoresis

DIPHTHERIA TOXIN

✓ Potent toxin = i.e it has a small lethal dose (0.1µg/kg)
✓ sole virulence factor → i.e responsible for Local effect & systemic complication
✓ β-phage encoded - gene is tox gene
✓ Iron level regulate the production of toxin
i.e when iron level is ≤0.1 mg/L → DT production ↑↑↑
✓ It's a A-B subunit toxin ; Mol. wt : 62,000 Da
✓ Receptor for DT → HB - EGF Precursor (Heparin binding Epidermal Growth factor)
✓ All cells are susceptible to action of DT EXCEPT RBC
✓ It's having affinity for M - Myocardium
A - Adrenals
N - Nerve endings

MOA: Receptor mediated endocytosis
↳ in endosome ⇒ A subunit seperate
↓
ADP Ribosylation of EF-2 ← Enters cytoplasm
↳ Inhibition of protein synthesis

✓ Strains used to prepare Diphtheria toxoid - Park william 8 Strain
↓
Add Formaldehyde ← take Filtarate ← Grown in broth culture
↓ leave it for 3-4 weeks
Formol toxoid → Adsorbed toxoid
Adsorbed on Aluminium Phosphate
↳ Acts as Adjuvent

Adsorbed toxoid is presenting in DPT Vaccine in a amount of 10-20 Lf units.

Schick Test :

- test done to check the susceptibility to diphtheria. No longer done
- It's a toxin neutralisation test

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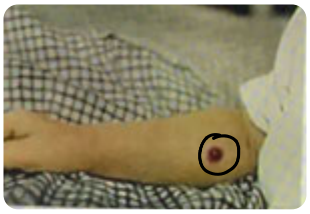
- done as an intradermal test
- Very small amount of diphtheria toxin injected to one forearm & inactivated diphtheria toxin is injected to othe forearm (act as a control)



↓

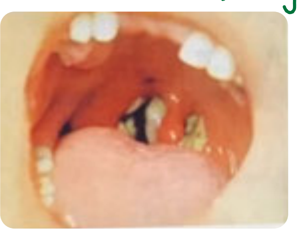
Look for Erythema or induration around 2-4th day

if Erythema present ⟶ +ve test ⟶ Susceptible to diphtheria.
if no Erythema ⟶ -ve test ⟶ not susceptible to dipht.
ie when you inoculated diphtheria toxin i/d, it got neutralised by antitoxin in blood.

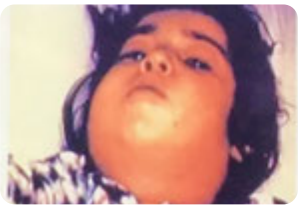
- Now we do
  ELISA on blood for Antitoxin level
  Protective titere of antitoxin level : 0.1 IU/ml of blood

Diphtheria

✓ Strictly human disease. ie Reservoir is Onlyhuman
✓ Mode of transmission : Secretion of cases or carriers through mucosa or skin
carrier can be in nose, throat (or) skin
✓ Peack age : 2-5yrs
✓ I.P : 3-4 days



MC type of diphtheria : FAUCIAL Diphtheria
(around the tonsil, formation of pseudomembrane)
present with : fever
Sorethroat
Pseudomembrane (Composed of tissue debris + WBC + RBC + Bacteria)
Cervical adonitis



✓ Sometimes a severe edema of Submandibular area, reflecting Severe inflammation of Submandibular region
BULL NECK
if untreated ⟶ death in 30%
MCC cause of death : Circulatory failure (due to Myocarditis)

✓ Other types:
- Laryngeal - Most dangerous ; requires immediate tracheostomy
- Cutaneous - caused by non toxic strain also.
- Nasal
- Otitic
- Conjuctival
- Vaginal

Complication :-

1. MYOCARDITIS : Most common (50% cases
   - around 7th-10th day AV block occurs
2. Neurological : due to DEMYELINATION
   - present as Palatine / Ciliary / Oculomotor paralysis
   - or leads to Paresis of extremities
   - appears around 10th -90th day
   - Slow complete spontaneous recovery occurs in all neurological complication

3. Hemorrhagic - Epistaxis, purpura
4. Obstructive - due to Pseudomembrain formation
 - mostly seen with Laryngeal diphtheria
5. Pneumonia
6. Otitis media

Diagnosis

✓ Sample : throat swab
✓ Stain : Gram & Albert
✓ Immunofluresence for Ag detection
✓ Culture : Loeflers serum slope/ Potassium tellurite

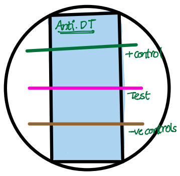
TOXIGENICITY TEST ;

(a) In vivo → Subcutaneous & i/d test done on Guinea pig & rabits
(b) In vitro

- Elek's Gel Precipitation Test :

- it's a immunodiffusion assay
- Double diffussion in 2 dimension

aka Ouchterlony Test



Other tests are

- Tissue Culture assay
- ELISA
- RPLA
- Cytotoxicity assay
- PCR for tox gene

Treatment

- Equine derived Anti DT →

if history < 3days old ⇒ 20-40000 Units (IM)
if moderate ≥ 3days ⇒ 80-120,000 Units (IM)
if severe ⇒ 80-120,000 Units (half im & half iv)

- Antibiotics → Pencillin/Erythromycin × 14 days
- Toxoid immunization
- Isolation of patient → Throughout t/t + till 2 consecutive swab culture (24hr) are -ve
- For carriers - Oral Erythromycin (7-10days)
 Or
 1 dose Benzathine pencillin (IM)
- Post exposure - same as above (for Non Immunised)
 ↳ if immunised in last 2yrs → Nothing to done
 ↳ > 2yrs → Single dose of Diphtheria toxoid

✓ C. pseudo tuberculosis & C. ulcerans can be infected by β-phage

C. pseudo tuberculosis ↓ Lymphadenitis in sheep

C. ulcerans ↓
- Acute pharyngitis
- But when it's infected with β-phage Human diphtheria also

Even these two species can produce diphtheria toxin.

metachromatin granule

- Diphtheriods
- Yersinia pestis
- B-pertussis
- Myco bacterial species
- Gardnella vaginalis

Ehrlich phenomenon :

- DT is a heat labile toxin.
- The discrepancy of the amount of antitoxin required to neutralise diphtheria toxin with time.
- As time ↑ DT amount ↓ [∵ it's heat labile]
 ∴ with time the amount of Antitoxin required to neutralise also get reduced.

Bacillus

- ✓ MC Lab contaminants of culture media
  ∵ spores are present everywhere in our enviornment.
- ✓ Gram positive rods
- ✓ Peritrichous flagellates } except B. antracis
- ✓ Non capsulated } except B. antracis
- ✓ Aerobes except B antracis (facultative anaerobes)

Spores

Non bulging
Heat & desiccasion resistant
Round / oval
Central / subterminal
Only in soil / culture
(Not in human body)

BACILLUS ANTHRACIS

- 1st pathogenic bacteria seen under microscope
- 1st pathogenic bacteria isolated in pure culture
- 1st pathogenic bacteria for which a live attenuated vaccine was made
- CATEGORY A (highest risk) bio terrorism agent.
- Largest pathogenic bacterium

General charachteristics:

- grows in the form of long chains
  Bamboo stick arrangement
- non motile
- Capsulated
- Facultative anaerobes

Culture:

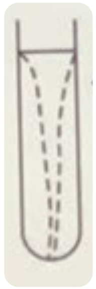
- Grow on simple/basal media
- Nutrient agar : Frosted glass appearance (with naked eye)
  Medusa head appearance (with a lens)
- Blood agar : Non-hemolytic
  but Anthracoid bacillus (other bacillus sp)
  ↓
  Hemolytic
- Selective medium: PLET medium
  Polymyxin Lysozyme EDTA Thallous acetate medium

Virulence factors:

1. Polypeptide capsule :-
   - polymer of D- glutamic acid
   - encoded by PXO2 plasmid.
   - demonstrated by MC Fadyean Reaction
     ⇓

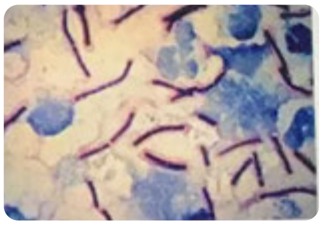
Biochemicals

- Catalase +ve
  Oxidase +ve
  Ferment sugurs
  Gelatin stab culture ⟶ Slow liquefaction
  ↓
  Inverted fir tree appearance/ Liquefaction



Special Charachters:

- String of pearl reaction ⇒
  On a medium with 0.5 μ/ml of pencillin
  ↓
  bacillus (rod shaped) becomes rounded
- Succeptible to γ phage
  ↓
  shows zone of lysis in colonies of B antracis
  Used to differentiate B. anthracis from Anthracoid bacillus



Smear of B. anthracis is taken
↓
Stain with Polychrome methylene blue
↓
Bacterium takes up the blue coloured stain
+
Pink coloured amorphous material around the blue bacterium (polypeptide capsule)

- it's Antiphagocytic

2. Anthrax toxin :
   - Encoded by PXO1 plasmid
   - Composed of 3 fractions (secreted seperately by bacterium) → 1. Edema Factor
     2. Lethal Factor
     3. Protctive Antigen (B subunit : binds to receptor)

Mechanism of anthrax toxin :

· 3 fractions are secreted seperately by Antigen
↓
Protective Ag [B subunit] binds to Anthrax toxin receptors on host cell
↓
Heptamarises ⟹ Protective Ag heptamer (PA heptamer)

→ Edema factor binds to PA heptamer
↓
Endocytosis ⟹ Edema toxin
↓
↑es CAMP (by calmodulin mediated mechanism) ⟶ CELL EDEMA

→ Lethal factor binds to PA heptamer
↓
Endocytosis ⟹ Lethal toxin
↓
Inhibits the activity of MAPKK's (enzymes) leads to CELL DEATH

ANTHRAX

- it's a zoonosis
- Reservoirs are Domestic animals : Vegetative bacteria in urine or faeces.
  ↓
  Sporulation in the envionment
- Spores are the infective form
- Infective dose may be small as 1 to 3 spores

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Types of Anthrax :

TYPES	SIGN & SYMPTOMS	COURSE	TREATMENT
1. Cutaneous Anthrax (through the break in skin) [Malignant pustules /Hide Porter's dis]	• Incubation Period ~ 7days • Painless pustule ↓ Ulcer	• Ulcer in 80-90% cases resolves spontaneously with Eschar formation • In 10-20% cases ↓ fatal septicemia	• Ciprofloxacin / Doxycycline (oral) ⇓ 7-10 days
2. Pulmonary Anthrax (due to inhalation of spores) [wool sorter's Disease]	• Hemorrhagic pneumonia (dyspnea, hemoptysis, Chest pain) • Pleuritis, mediastinitis/ meningitis may also be associated with Pneumonia	• In 80-90% cases of untreated ↓ Fatal septicemia	• Ciprofloxacin + Meropenem + Clindamycin / Linezolid Rifampicin ⇓ for 60 days
3. Intestinal Anthrax (rare type) (due to ingestion of poorly cooked meat) ↓ heat resistant spores present	• Hemorrhagic Enteritis	• In 25- 60% cases ↓ Septicemia	

Diagnosis:-

• Specimen used	Exudate/Swab (Cutaneous anthrax) Sputum Stool
• Gram staining	Only gram +ve rods (no spore)
• Antigen detection	Immunofluorecence
• Culture	Only in BSL-III Lab
• Serology	ELISA/Complement fixation /indirect fluroscent Ab Test ↓ Ab will be detectable around 7th-10th day of illness

• Molecular methods: PCR/Nucleic acid probe (most sensitive)

• Experimental animal used : Shaved skin of Guinea pig

Vaccines :

- Subunit vaccine
- It's a culture supernatant known as : Anthrax vaccine Adsorbed
- Strain used : STERNE strain
  ↓
  Non capsulated toxigenic strain
- Supernatant of broth suspension
  ↓
  Contains a lot of Protective antigen
  ↓
  ∴ induces protective Antibody
- 5 dose regimen + Booster every year for continous protection
- Post exposure Prophylaxis
  - Doxycycline
    or
  - Ciprofloxacin/ Levofloxacin
  - Amoxicillin

  } Should be given for 60 days.

Bacillus Cereus

- non-capsulated
- shows swarming on blood agar
  hemolytic in blood agar
- Selective medium : MYPA (Mannitol Egg Yolk Phenol Red Polymyxin Agar)
- Spores of bacillus cereus are present everywhere (Soil, water, vegetables, cereals, pulses)
- Causes food poisoning
  are of 2 types:

	EMETIC TYPE	DIARRHEAL TYPE
Serotypes	• 1, 3, 5	• 2, 6, 8, 9, 10, 12
foods	• Fried /Chinese rice	• Poorly cooked meat with heat resistant spores
toxins	• CERULIDE : Preformed toxin ↓ causes Vagal stimulation similar to staph. aureus Enterotoxin	• NHE toxin : Formed in GIT secretion of ions ↓ secretory diarrhea similar to labile toxin of ETEC & Cl. perfringes enterotoxin
incubation period	• 1-6 hrs	• 8-16 hrs
Clinical feature	• N,V, Abdominal cramps & wattery diarrhea	• Abdominal cramps & watery diarrhea
Course	• Self limiting disease	• Self limiting disease

T/t : Fluids & electrolyte supplementation. No antibiotics

Clostridium perfringens and C.tetani

✓ Closter means spindle (due to bulging spore → spindle shape)

General Charachteristics

- highly pleomorphic gram +ve bacilli
- Peritrichous flagellates except
  C. perfringes & C. teni VI
- Non capsulated except
  C. perfringes & C. butyricum
- Strict anaerobes except
  ↓ C. histolyticum (aerotolerant)
  ∵ they lack enzymes that removes O_2 free radicals
  ie Catalase, peroxidase, superoxide dismutase
- Soil saprophytes
- GI Commensal flora
  (C. perfringes, tetani, ramosum)

Spores :

- Bulging spores
- Can form in soil, culture + human body
  (except C. perfringes)
- Sporulate very easily Eg: C. sporogenes
- Perfringes forms spores only in Ellner's medium
- killed within 5-10 min of boiling
  Exception: C. botulinum
  C. perfringes
- all are killed by Autoclaving

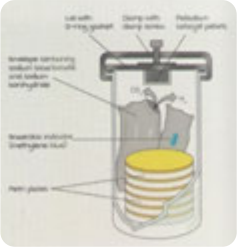
Position of spores:

1. Subterminal spores → C. perfringes, C. histolyticum, C. septicum, novyi, C. botulinum } Gas gangrene
2. Terminal + Circular (Drum stick appearance) → C. tetani, tetanomorphum, sphenoides
3. Terminal + Oval (Tennis racquet appearance) → C. difficile, tertium, C. cochlearum
4. Central spores → C. bifermentans

Cultivation

2 requirements for growth:

1. Low redox potential : Thioglycollate (transport medium)
   Mettalic iron
   Unsaturated fatty acids (Robertson's cooked meat broth)
2. Strict anaerobosis : by
   1. Anaerobic / mac intosh Filde's jar
   2. Anaerobic gas packs (now we use)
   3. Sterile petroleum jelly



- medium most commonly used for isolation of clostridia

↓

Robertson's cooked meat broth (RCM broth)

↓

Contains nutrient broth with meat particles (low redox)

↓

Above this sterile petroleum (Prevent diffusion of O_2) jelly is poured

- based on the growth in RCM broth

Proteolytic (Proteins broken down)
↓
Meats turns black
- Predominantly → Sporogens, sordellii, C. histolyticum, Biofermantans
- Mildly → C. tetani

Saccharolytic [Sugars → broken down]
↓
Meat turns pink
- Predominantly → C. perfringens, difficille, novyi, Septicum
- Only saccharolytic → C. botulinum C, D, E

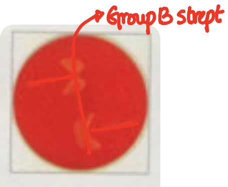
Other medias for Clostridia:

- ✱ Blood agar
- ✱ Selective media : Blood agar with Neomycin/Polymyxin
- ✱ PRAS Media : Enzyme based (Oxyrase) technology
  (Pre reduced Anaerobically Sterilised)

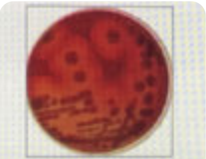
C. PERFRINGENS (C. WELCHII)

- Non motile
- Capsulated
- Subterminal spores
- Can grow upto 45°C

• Litmus milk : Stormy fermentation



• Reverse CAMP test +ve (Bow tie appearance)



• Blood Agar : Target hemolysis

Each colony surrounded by complete hemolysis & further surrounded incomplete hemolysis

Toxins

12 histolytic toxins

Major toxins
- α, β, ε, ι
- Based on the spectrum of β, ε, ι based on C.perfringens is divided in to 5 types ⇒ A to E

Minor toxins
- θ, ?, ζ

α - toxin :

- most important toxin of C. perfringens
- It has Lethal, Dermonecrotic & hemolytic (Hot - cold type) activity
- It has Lecithinase activity → demonstrated by

↓

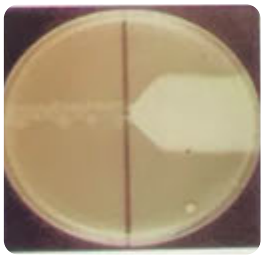
NAGLER REACTION (neutralisation test)

↓

Egg yolk agar (contain lecithin)

⇓

divided into 2 halves



One half → Anti α toxin → Streak Inoculation of Cl. colonies → Break down of lecithin (due to α toxin) → Neutralised by anti α toxin

Other half → Plain medium → Streak Inoculation of Cl. colonies → α toxin produced (breakdown of lecithin) → Zone of opalescence

- all strains of Cl. perfringens → 100% +ve for α toxin

Other toxins :

* β, ι, ε → Dermonecrotic & lethal particles

* θ toxin → Perfringiolysin (Oxygen labile hemolysins)

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GAS GANGRENE

- also known as Anaerobic myonecrosis
- most important infection caused by C. perfringes
- seen in RTA, war wounds
- Most important toxin responsible for tissue destruction

↓

α toxin

- Lecithinase activity
- Aggregation of platelet
- Intravascular Hemolysis

- Other toxin causing tissue destruction : θ toxin
- I.P : 12-36 hrs.
- Diagnosis :
  - Specimen ⟶ exudates, tissue bits from wounds
  - Gram smear ⟶ BOX CAR APPEARANCE
  - Cultivation ⟶ Anaerobic conditions
- Treatment
  - Surgical debridement
  - Parenteral antibiotics ⟶ Pencillin + Clindamycin
  - Hyper baric Oxygen
- Other causes of gas gangrene :
  - C. novyi
  - C. septicum
  - C. histolyticum
  - C. sordelli
  - C. fallax

FOOD POISONING

- due to meat containing heat resistance (type A) of C. perfringens
- I.P : 8-24 hrs
- C. perfringes enterotoxin (CpE) is formed in GIT

↓

Fluid accumulation by pore formation & secretion of ions

- C/O : Abdominal cramps with watery diarrhea (self resolving)
- Diagnosis :
  - toxin detection by ELISA/tissue culture/PCR for CPE gene
  - Stool culture : $>10^6$ C. perfringens/g of stool
  - Food culture : $>10^6$/g
- Treatment symptomatic (self resolving)

PIGBEL/NECROTISING ENTERITIS

- due to Type C strain of Cl. perfringes (heat resistant spore)
- β-toxin is responsible
- Food implicated ⟶ Spore contaminated PORK (especially if ingested with trypsin inhibitors like sweet potato)

↓

Prevent break down of β-toxin

- Patient present with bloody diarrhea
- High mortality rate
- Treatment : Parenteral Pencillin + Metronidazole

C. TETANI

✓ 1 to X̄ serotypes (Antigenic types)
✓ motile except VI
✓ non capsulated
✓ Drumstick appearance : Terminal, circular spores
✓ Swarming ⊕ ⇒ used in FILDE's technique

↓

for isolation of C. tetani from other C. species

take a BA slant ⟶ inoculate sample at bottom of slant ⟶ incubate in anaerobic conditions

because of swarming it will climb over the slope ⟶ subculture from top of slant.

Special charachters :

⟶ Neutral Red medium : colonics shows fluorescence under UV light

⟶ Gelatin stab culture : Fir tree appearance

TOXINS:

-plasmid mediated

1. Tetanolysin : Oxygen labile hemolysin
   no role in tetanus
2. Tetanoplasmin: Sole virulence factor of C. tetani
   secreted in the form of Protoxin
   ↓ Proteases

Axonal transport ← L chain-S-S-Heavy chain
↓
Site: Ventral horns of spinal cord
Motor nuclei of cranial nerve

MOA: Presynaptic inhibition of release of inhibitory NT (GABA + Glycine)

Toxin cleaves Synaptobrevin (VAMP)
↓ functions as
fusion of vesicles of GABA + Glycine with neuronal cell membrane
↓
This presynaptic inhibition of release of GABA + Glycine
↓
Uncontrolled stimulation of motor neurons
⇓
Rigidity + spasms

TETANUS/LOCK JAW

- mostly in hot + damp climate
- infective form : Spores
- I.P : 6-12 days (upto 12 weeks)
  shorter the I.P ⟶ severity ↑ses.
- MC : Generalised tetanus

Initially ⇒ Trismus (lock jaw)
↓
Risus sardonicus
(spasms of orbicularis oris)
↓
Ophisthotonus, neck, rigidity

- mortality → 15-50%.
- Other types of tetanus:
  - Cephalic tetanus
    (following otitis media/head injury)
  - Localised tetanus
  - Uterine tetanus } High mortality
  - Neonatal tetanus } High mortality
    (8th day disease)
- Diagnosis:
  - mostly clinical
  - swabs ⟶
    - * Gram staining : Gram +ve bacilli
    - * Culture : Strict anaerobic conditions on blood agar
    - * mouse bio assay
- Treatment:
  - Human tetanus immunoglobin 500 units i/m or i/v (to neutralize the toxin that is not yet bound to receptors)
  - Metronidazole or Pencillin IV
  - Quiet enviornment
  - Muscle relaxants
  - Sedation
  - Respiratory + Nutritional support
  - Once the patient recovers → full course of tetanus toxoid
- Prophylaxis:
  - Tetanus toxoid → fluid toxoid prepared by formalin
    ⇓
    Adsorbed onto Aluminium salts
  - 100% efficasy
  - Protective titre of antitoxin → >0.1 μ/ml of serum

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Clostridium botulinum AND C.difficile

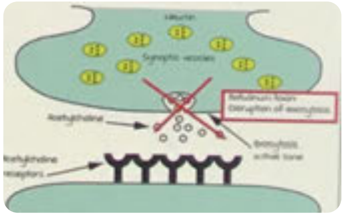
C. botulinum

- ✓ motile member
- ✓ Noncapsulated
- ✓ Subterminal spore
- ✓ Strict anaerobe

Botulinum toxin

- Category A bioterrorism agent
- Most potent toxin → Minimum Lethal dose : 1-2 ng/kg
- It's released on lysis of bacteria; not secreted
- 9 antigenic types ⇒ A, B, C1, C2, D, E, F, G, H
  - C2 ↓ Enterotoxin
  - H ↓ Newly added
- All others except C_2 are Neurotoxin
- Most common cause of human botulism : A, B, E
- All of them are chromosomal mediated except C&D (phage mediated)
- Most severe disease is caused by : A
- Other species secreting this toxin : C. butyricum
  C. barati
  C. argentinense
- Released as a PROTOXIN $\xrightarrow{\text{Protease}}$ L-chain - s - s - Heavy chain
  - L-chain ↓ Active part / A subunit
  - Heavy chain ↓ Binding part / B subunit
  - ↓ Absorbed in to blood & lymph → Site of action :
    - Neuromuscular junction
    - Autonomic ganglia
    - Parasympathetic post ganglionic terminal
    - Sympathetic post ganglionic terminals

✓ MOA : Presynaptic inhibition of Ach release



by proteolysis of snare proteins
↓
it's responsible for fusion of ACh vesicle to neuronal membrane.

Snare proteins include : Synaptobrevin → B, D, F, G, H
Syntaxin → C1
SNAP-25 → A, E

Food borne Botulism

- Home preserved food (MC) } contains
  Industrialised canned food } PREFORMED toxin
- It's a heat labile toxin
- I.P : 12-36 hrs
- Clinical feature:

→ Initially nausea & thirst (action on ANS)
↓
→ Palsies of cranial nerves :
  - Blurring of vision/diplopia
  - Dysphagia, dysarthria, dialated pupil
  - Descending Symmetric flaccid paralysis

→ Constipation
→ No fever
  No sensory deficit
  No altered mentation
→ Mortality → Respiratory paralysis
  → 25-70%.

Uses of Botulinum toxins:

- Blepharospasm
- Achalasia
- Strabismus
- Tics
- Myoclonus
- Dystonia

} Effect starts in 1-2 wks
↓
Last for 10-12 wk

* most commonly used toxin: A & B

Infant botulism

- Most common type of botulism
- Cause : Ingestion of spore contaminated honey/ infant milk powder
- 1st symptom : Constipation
- Floppiness, weak cry, poor feeding
- Descending symmetric flaccid paralysis
- Mortality : ~1%.
- Also called as Floppy Baby syndrome

Wound botulism

- Rare type
- Wound contaminated by spores
- IV drug abusers who administer Heroine intradermally
- I.P ⟹ 7 to 10 days
- Symptoms are same
- GI symptoms :
  Thirst + constipation = ABSENT
- Diagnosis
  - Demonstration of toxin food/stool/serum
    - by ELISA, IF, Tissue culture, PCR
  - Culture
  - Mouse bioassay
- Treatment
  - Antitoxin - either human/equine derive
  - Respiratory support
  - Nutritional support
  - Recovery → Due to sprouting of new axon terminals
    → takes several months

C. difficile

- motile • non-capsulated
- Tennis Racquet appearance → Due to terminal oval spores
- Normal GI Flora → 2-3% of adults
  → 50-70% of infants & children

6-8% strains ⟹ Toxigenic → Toxin A & Toxin B (Tcd A & Tcd B)

- Mechanism of action : Toxins (Glucosyl transferase)

Glucosylate GTPase (Cdc 42, Rho, Rac)
↓
- Actin polymerization
- Cytoskeletal architecture maintaince
↓
Cytoskeletal de arrangement → mucosal injury + inflammation

↑ IL-8 secretion
⇓
Recruitment of neutrophil

Pathogenesis of AAD & PMC

AAD : Antibiotic associated diarrhea
PMC : Pseudomembranous colitis

History of ingestion of broad spectrum antibiotics
↓
Disruption of normal colonic flora
↓
Colonization by Toxigenic C. difficile
↓
Toxin A & D

Antibiotic that are implicated :
- 2nd + 3rd gen cephalosporin
- Ampicillin / Amoxicillin
- Clindamycin

Now : FQs, Macrolides

Clinical features :
- watery diarrhea (<10 stool/day)
- Blood in stool - very rare
- Fever 25%
- Abdominal cramps
- **Fulminant disease** → Toxic megacolon
  Adynamic ileus
  Intestinal perforation
  Peritonitis.

Diagnosis & Treatment

(1) Colonoscopy :
- Yellow-white plaques / Pseudomembrane covering an erythematous mucosa
- 100% specificity

(2) Detection of toxin in stool
- Tissue culture / Cytotoxicity Assay
  ⇒ Gold standard
  ⇒ 97-99% specific
- ELISA (rapid) ⇒ 93-99% specific
- PCR ⇒ 88-97% specific

(3) C. difficile detection in stool
- Culture on CCFA medium → Gold standard
  - CCFA : Cycloserine Cefoxitin Fructose Agar
  - 96-100% Specificity
  → **No differenciation b/w toxic & non toxic strains**
- Glutamate dehydrogenase - ELISA
  → **No differenciation b/w toxic & non toxic strains**

Treatment

(1) Mid/moderate disease : Oral metronidazole
(2) Severe i.e WBC > 15,000 μL
Creatinine : 1.5 x normal
treatment : Oral vancomycin
(3) Fulminant ⟹ Oral vancomycin + iv Metronidazole

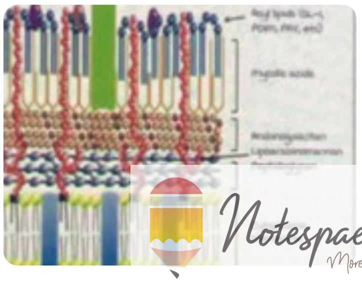
Mycobacteria

CRITERIA

① ACID FAST
② High G+C content in genome (60-70%)
③ Mycolic acid in cell wall

General Characteristics

- ✓ Gram +ve bacilli
- ✓ **Gram stain can not penetrate the mycobacteria** because of the presence of several complex lipid like Acyl lipids, Mycolic acids, Arabinogalactan, lipoarabino mannan
- ✓ Non motile
- ✓ Non sporing
- ✓ Non capsulated
- ✓ Strict aerobes or **micro aerophilic** (can tolerate upto 2-8% of O_2 level)



ATYPICAL BACTERIA

- ✓ Also known as tuberculoid mycobacteria or Non-tubercle mycobacteria or MOTT
- ✓ Also called as Enviornmental mycobacteria because reservoir is soil & water
- ✓ No human to human transmission
- ✓ mainly cause disease in individuals with Deficient CMI
- ✓ negative Influence on immune responce to BCG Vaccination
- ✓ Low grade tuberculin reaction
- ✓ False positive sputum smears as they are acid fast with same 20% sulphuric acid as MTB How to overcome is this?

  NOT ALCOHOL FAST (M.T.B - Both acid & alcohol fast)

  When 3% alcohol is used, M.T.B Retains the color, where as Atypical mycobacteria get decolourised.

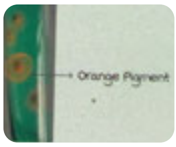
Classification of Atypical Bacteria

✓ RUNYON'S CLASSIFICATION: According to the rate of growth & pigment production

- Photo chromagens: Produce pigment only in light.
  Eg: M. kansasi, M. marinum, M. simiae, M. asiatum
- Scoto chromagens: Produce pigment in DARK
  Eg: M. szulgai, M. scrofulaceum, M. gordanae
- Non photo chromagens: NEVER produce pigment
  Eg: MAC, M. ulcerans, M. xenopi, M. genavense
- Rapid growers: Form colonies in 5-7 days. Can be pigmented/Non pigmented
  Eg: M. fortuitum, M. cheloni, M. abscessus, M. phlei, M. vaccae, M. smegmatis

Cultivation:

(1) LJ Medium:
- Form colonies in 3-8 wks
  5-7 days (if rapid growers)
- ORANGE pigment is produced



Biochemicals:

- All are catalase +ve
- Peroxidase -ve
- Arylsulfatase +ve
- Niacin test -ve

Infections by Atypical Bacteria:

- Any species of Atypical mycobacterium can cause any of the diseases below:

(1) Pulmonary disease resembling T.B
- if CMI is less, Disseminated disease
- MCC: MAC
- 2nd MCC: M. kansasi

(2) Lymphadenitis
- MCC: MAC - 2nd MCC: Scrofulaceum

(3) Skin & soft tissue infection:

(a) Post traumatic/surgical/injection abcess
- MCC: Rapid growers

(b) Swimming pool/Fish tank granuloma
- caused by M. marinum (Fish tubercle bacilli)
- Sporotrichoid spread +/-

(4) Buruli ulcer
- only caused by M. ulcerans

Treatment

- most resistant to antitubercular drug
- MAC/Kansasii disease - combined INH + Rifampcin + Ethambutol
- Others: Rifabutin, Clofazimine, FQ's, Newer macrolides - effective in combination used only after sensitivity testing

---

- Battey's Bacillus: M. intracellulare
  Tap water scotochromagen: M. gordanae
- Only species producing toxin MYCOLACTONE: M. ulcerans
- Species with immunomodulating property: M. vaccae, M.w / M. indicus pranii
  this vaccine is used as prophylaxis against Leprosy in India

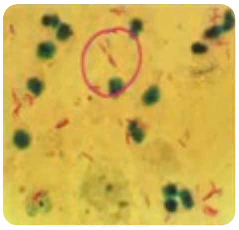
M. TUBERCULOSIS COMPLEX

- Species which is scoto at 37°C & photo chromogen at room temp: M. szulgai
- Species which requires Mycobactin-J in LJ Medium : M. genavense
- Species which require Hemin:- M. hemophilum

✓ >99.9% DNA homology

SPECIES	RESERVOIR
M. tuburculosis	Human
M. africanum	Human
M. canetii	Human
M. bovis	Cattle
M. caprae	Cattle
M. microti	Voles
M. pinnipedii	Seals & Sealions
M. orygis	Oryxes
M. mungi	Banded mongoose
M. surricatae	Meer cats

M. bovis to M. surricatae: ZOONOTIC TB IN HUMAN



Diagnostic methods of T.B :

1. Microscopic
   - ZN stain
   - Kinyown /Cold/ Gabett stain - Concentration of phenol in carbolfuschin is increased, so no need of Heating.

   Sensitivity : 10^4 bacilli/ml
   - fluorescent dyes : Auramine O & Rhodamine B - binds to mycolic acid & shows fluorescence

     Advantage : Better sensitivity than routine microscopy
Faster

     Disadvantage : No differenciation of live from dead
Poor sensitivity in comparison to culture

Biochemical Reactions for M.tuberculosis

	M.tb	M. bovis	MOTT
Catalase	weak +	weak +	+++
Peroxidase	+	+	−
Arysulfatase	−	−	+
Niacin	+	−	−
Nitrate reductase	+	−	
Pyrazinamidase	+	−	
TCH Sensitivity	Resistant	Sensitive	

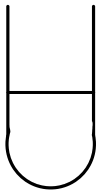
Catalase to Niacin: M.tb from MOTT

Niacin to TCH Sensitivity: M.tb from M.bovis

✓ Niacin test is positive for M. simiae & M. chelonae
✓ strains resistant to INH (due to KAT G mutation) - Catalase, Peroxidase Negative
✓ Neutral Red test +ve only for virulent strains of M.tb

Homogenization and Decontamination :

- Recommended method by W.H.O - Petroff's method (4% NaOH)



Sputum + 4% NaOH → manual shaking 15-20 min → Centrifuge @ 3000 rpm 15-20 min → Discard supernatant in 5% phenol → Take lower 1ml of sediment

Neutralise alkali by N/10 HCl

After this Specimen is ready for culture + Animal pathogenicity
- Apart from 4% NaOH, we can use 2% NaOH + N-acetyl-L-cysteine

CULTIVATION

- ✓ Culture: Gold standard for diagnosis
- ✓ Sensitivity: 10-100 bacteria/ml
- ✓ M.tb is obligate aerobe
- ✓ Generation time: 14-15 hours
- ✓ 0.5% Glycerol enhances the growth of M.tb & inhibits m.bovis
- ✓ Sodium pyruvate: enhance the growth of both M.tb & M.bovis

Culture media:

Solid:
- Egg containing:
  - LJ
  - Dorset egg medium - Lack glycerol
  - Petragnini
- Blood containing: TARSHI
- Serum containg: Loeffler
- Potato containg: Pawlowsky
- Agar: middle broke 7H10 or 7H11
- Colonies of M.tb:
  - Eugonic:- Rough - tough - buff
- Colonies of M.bovis
  - Dysgonic:- Smooth - shiny - white
- Colonies appear in 3-8 weeks

Liquid:
- Rapid growth in comparison to solid media
  - Eg: - Middle broke 7H9, 7H12
  - Sula
  - Sauton
  - Dubo
  - Proskauer & Beck
- In liquid media
  - Serpentine cords of growth in virulent strain due to cell wall factor CORD FACTOR help in intracellular survival

Rapid methods for Diagnosing Tuberculosis:

- Also known as Automated Methods
- Turnover time: 8-14 days.

NOTE: PANTA is a antibiotic mocktail

1. BACTEC 460 TB:
- Automated Radiometric method
- MB 7H12 + PANTA + Radiolabelled palmitic acid

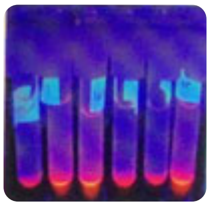
↓

Sputum +ve → Measured by Scintillation counter

↓

Expressed as Growth index

2. BACTEC MGIT



- Automated fluorometric method
- MB 7H12 + PANTA + Ruthenium

↓

Sputum +ve specimen

↓

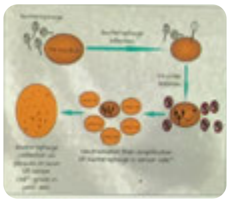
Ruthenium will utilise O_2. ↓ it concentration

↓

Produce fluorescence

3. Mycobacterium phage based assay:

FAST Plaque TB: These are viruses which infect M.tb
- Sensor:
  - M.smegmatis



4. BACT Alert
- Automated Calorimetric method

MB 7H12 + PANTA + Substrate/Sensor which changes colour with increasing CO_2.

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MOLECULAR METHODS

- nuclic acid probes
- NAAT - PCR, LCR, TMA, SDA, Realtime PCR
- Detect even if 1 bacterium/ml
- Highly specific
- most commonly used target: insertion sequence IS6110

Animal pathogenicity:

- Guinea pig - for Mtb & M.bovis
- Rabbit - only for M.bovis

DRUG RESISTANCE

1. Primary / Transmitted Drug Ⓡ : Infection due to drug Resistant strain
2. Secondary / Acquired Drug Ⓡ : Ⓡ developed during t/t.
3. Monodrug Resistance : Ⓡ to one ONE 1st line ATP (HRZES)
4. Polydrug Resistance : Ⓡ to >1 1st line ATP other than H & R
5. Multidrug Resistance : Ⓡ to both INH & Rifampicin
6. Extensively Drug Resistance (XDR) : MDR + FQ's + One of the injetable 2nd line ATD
7. Total Drug Resistance / XXDR : Resistance to all 1st & 2nd line ATD

✓ Resistance is due to : Gene mutation
- INH Resistance is due to gene mutations of Inh A, KatG, AhpC
- Rifampicin Resistance : rpoB gene mutations

Methods of Sensitivity testing in M.tb

- Disc diffussion method is never used (Stability of disc ↓ with long incubation)
- Conventional method : LJ medium with ATD's

a. Absolute concentration method :

- LJ medium with serial concentration of ATD's
  +
  Standard inoculum of test
  ⇓
  MIC of test strain compared to MIC of lab strain

b. Resistance Ratio method

- 2 sets of LJ media with serial conc. of ATD's
- In 1 set : Add standard inoculum of test bacterium
  2nd set : Add. standard inoculum of lab. strain - H37RV
- Determine MIC of both & Ratio will tell about the resistance

c. Proportion method

- WHO recommended
- 2 sets of LJ media -
  one is Drug free
  second is fixed drug conc } Add serial dilution of Std. inoculum
  ⇓
  Count no. of colonies on drug free and these will be compared with drug containing

d. Others

- Automated methods
- Phage based assays
- MODS (Microscopic Observation of drug susceptibility) - Done in a liquid medium look for turbidity, takes 5-10 days
- Molecular methods : Gene mutations detected
  - MTB / Rif assay or Xpert MTB assay
    - Real time PCR based method
    - Takes 90-120 min
    - Amplifying IS6110, also rpoB gene mutation

INF-γ Release assay:

- Detects exposure to tubercular antigen only due to INFECTION, not due to vaccination

Patient whole blood + Add Antigen → Measure IFN-γ released from the sensitised T cell
Measured by ELISA

↓
CFP-10
ESAT-6
TB-7.7

- Montoux test done early, detect tubercular Ag due to BCG vaccine & infection.

MPB-64 PATCH TEST:

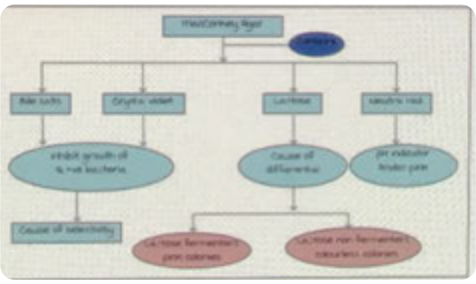
- MPB-64-Ag extract of M. bovis. BCG-64.
  Applied as patch on forearm → look for Induration
- Distinguish active from latent TB with a sensitivity of 98% and specificity of 100%.

LAM ASSAY

- Lipoarabinomannan assay
  - Ag released from cell wall of metabolically active & degenerating M.tb
  - Detect in urine to determine Active v/s laten infection
- Good sensitivity only in HIV patient with Active TB.

Enterobacteriaceae-1

✓ Contain 40 genera
✓ Gram negative bacilli
✓ Non sporing, facultative anaerobes
✓ Motile by Peritrichous flagellae
except: Klebsiella, Shigella, Y. pestis, Salmonella gallinarum, Pullorum; Atypical E. Coli - Non motile
✓ All are catalase +ve except Shigalla dysnteriae type I
✓ Oxidase -ve
✓ All reduce Nitrates to Nitrites
✓ Ferment sugar → if produce only acid → Anaerogenic
if produce acid + CO_2 → Aerogenic
CO_2 production seen in Durham's Tube
✓ They can grow on simple media
✓ On MacConkey - Lactose fermenting → PINK Colonies
- Non Lactose fermenting → PALE Colonies
✓ Ingradient of MacConkey medium: Bile salts + Crystal violet + Lactose + Na. taurocholate + Neutral Red + Agar + Peptone
✓ Mac Conkey : Selective, Differential, Indicator medium



Lactose fermenters:

- E. Coli
- Klebsiella
- Citrobacter
- Enterobacter

Non-Lactose fermenters:

- Shigella
- Proteus
- Morganella
- Salmonella
- Providencia
- Yersinia

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✓ Members of Enterobacteriacea:
- Escherichia, Shigella, Edwardsiella, Citrobacter, Klebsiella, Enterobacter, Hafnia, Serratia, Salmonella, Yersinia
- Proteus, morganella, Providencia - PPA test +ve

SHIGELLA

✓ Non motile, noncapsulated
✓ On the basis of O Ag / Somatic Ag divided in to 4 species

Biochemical reactions of Shigella:
- Anaerogenic except some S. flexneri & S. boydii
- NLF, MF
- On sugar fermentation - H_2S -ve
- IMViC ⟶ - + - -
- Urease ⟶ -ve

Biochemical Test:
1. Indole test: Ability of bacterium to convert tryptophan ⟶ indole
2. Methyl Red test: On glucose utilisation → Acid ↑↑ (pH<4)
3. Voges proskauer: On glucose utilisation ⟶ Acetoin production detected
4. Citrate: Ability to utilise citrate as the sole source of carbon

	SEROTYPES	MANNITOL FERMEN	LACTOSE FERM.	SPECIAL COMMENT
S. dysentriae	10 1 - Shiga's Bacillus	Non-MF	Non-LF	Most severe
S. flexneri	6	MF	Non-LF	MC in India
S. boydii	~19	MF	Non-LF	Least common
S. sonnei	1	MF	Late LF	• mildest disease • MC species in west • MC in world • Most resistant to chemical disinfectant.

Culture:
- On MacConkey: NLF Colonies
- Transport - Sach's buffered Glycerol saline, Cary Blair medium
- Enrichment - Selenite F broth, Gram negative broth
- Selective - DCA, XLD, SSA, HEA (Hektoen Enteric agar)

BACILLARY DYSENTRY

- Shigella causes Invasive diarrheas
- Site: Colon rectal mucosa
- Invasion because of Virulence plasmid
- Virulence plasmid produces 2 virulence factor - Type III Secretion system
  Invasive Plasmid Ag (or)
  Virulence marker Ag
- Invasion is BASOLATERAL, not apical due to bacterial mediated endocytosis.
- Shiga toxin is produced only by Shigallae dysentriae type 1. It plays a supplemary role in the disease.
  - It's a AB5 subunit toxin
  - Toxin receptor: GB3
  - Site of action: Endothelial cell of small blood vessels of GIT

- **MOA** : Toxin binds to Gb3 receptor

↓

Endocytosis of toxin

↓

A & B unit's get seperated A → $A_1 + A_2$

A_1 enters cytoplasm & cleaves 28 s rRNA of 60s ribosome Subunit

↓

Inhibition of protein synthesis

↓

Necrosis of endothelial cell

↓

Activation of coagulation & creates local ischaemia

- In 5-10% of S. dy's I → Hematogenous dissemination → induce necrosis of glomerular capillary endothelial cell → HUS (Hemolytic Uremic Syndrome)
- MCC of HUS in India : Shigallae dysentriae type I
  MCC of HUS in world : EHCC

SHIGELLOSIS

- Strict human disease
- I.P : 12 - 5 days
- Infective dose : 10-100 bacteria, fomites play important role in transmission
- Person to person transmission, flies, contaminated food & water
- Patient complains of fever, Abdominal cramps, Diarrhea f/b dysentry

Complication

- Toxic megacolon
- Intussusception
- Reactive arthritis (seen in flexneri)
- HUS (S. dyn type-1)

Treatment

- Rehydration + Antibiotics (Cipro)
- Alternative - Ceftriaxone / Azithromycin
- R-plasmid are common in Shigella - Intrinsic resistance to Pencillin

Diagnosis :

- Fresh stool of pt → Gram smear
  Pus cells +++
  RBC +++
- ELISA for VMA's
- Prove invasiveness -
  (i) Sereny's test - Kerato conjuctivitis in guinea pig / Rabbit
  (ii) Invasion of HeLa / Hep2 cell lines

ESCHERICHIA COLI

- motile except Atypical
- 80% capsulated
- Serotyping - O Ag - >170 'O' serotypes
  K Ag - >100 'K' serotypes
  H Ag - > 75 'H' serotypes

✓ E. Coli present as a Normal Gut flora

✓ It's MC facultative Anaerobe present in human GUT

Biochemical Reaction :

- Aerogenic on sugar fermentation
- LF, SF, MF
- Do not produce H_2S
- IMViC + + - -
- Urease -ve

CULTURE

- On Blood Agar - β hemolytic
- On MacConkey - LF

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DIARRHEAGENIC E.COLI

- These are NEVER Normal flora

Entero invasive E.Coli (EIEC)

- Belong to Atypical E.Coli i.e they are Non-motile, Non-lactose fermenting, Anaerogenic
- Causes disease similar to shigella with 2 differences
  - (i) Infective dose is ~1000 times larger than shigella
  - (ii) Non of them is producing shiga toxin

Enterotoxigenic E.Coli (ETEC)

- MC cause of traveller's diarrhea
- Diarrhea in all agegroups.
- Infective dose is $>10^9$
- Only transmission by contaminated water/food

VIRULENCE FACTORS:

due to the presence of virulence plasmid

(i) Colonisation factor Ags
- present in fimbriae/outermembrane
- mediates Adhesion
- Eg: CFA-I, CFA II, CS-1, CS-2

(ii) Toxins: Heat labile & Heat stable toxin

HEAT LABILE TOXIN	HEAT STABLE TOXIN
- similar to cholera toxin	- 19 aminoacids long polypeptide
- AB5 subunit toxin	- MOA: ↑cGMP → by directly increasing the activity of Guanyl Cyclase
- MOA: ↑cAMP (by ADP ribosylation of Gs α)	

Both causes secretions of ions → Secretory Diarrhea

- Patient with Abundant watery diarrhea, Abdominal cramps, fever & vomitting
- Usually self: limitting in 3-4 days

Diagnosis:

(i) Gramstaining - NO PUS Cells/RBC - not an invasive diarrhea

(ii) Cultivation: On macConkey

(iii) Prove it as ETEC - Toxigenicity tests

FOR LABILE TOXIN	FOR STABLE TOXIN
- Rabbit ileal loop Assay (read @ 18 hrs)	- Rabbit ileal loop Assay (read @ 6hrs)
- Biken test - immune diffussion test	- Infant (suckling) mouse intragastric assay
- Y1 Adrenal tumor assay	- ELISA/RIA
- ELISA/ RIA - toxin detection	- PCR/ Probes
- PCR /Probes - Gene detection	

Treatment: fluids & electrolytes & FQs. (Fluoroquinalones)

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ENTEROPATHOGENIC (EPEC) E.COLI

- MCC of infantile bacterial diarrheas
- Rarely causes disease in Adults
- Virulence Plasmids :
  - EPEC Adherence factor plasmid → Bundle forming pilus → Adherance
  - Locus of Enterocyte Effasement plasmid → Attachement with cuplike pedestels with effasement of villi

↓

A.E effect (Attachement Effasement)

↓

Secretory Diarrhea

- Baby presents with watery Diarrhea
- DIAGNOSIS :
  (i) Culture on MacConkey
  (ii) Prove it's EPEC : Hep2 Adherance assay

↓

Localised Adherance pattern

ENTERO AGGREGATIVE E.COLI (EAEC)

- MCC cause of Persistent Diarrhea
- it can cause Traveller's Diarrhea
- Secretary diarrhea
- Hep2 Adherance assay ⇒ Stalked Brick Adherance pattern

ENTEROHEAMORRAGIC E.COLI (EHEC)

- Also called as Verotoxigenic E.Coli
- MCC of HUS in the world
- Reservoir : GIT of cows (zoonotic)
- MC food history : Poorly cooked beef / Hamburgers / Contaminated salads, Water, fomites, person to person
- Infective dose : 10-100
- Virulence factors :
  (i) Locus of Enterocyte effasement plasmid
  (ii) Phage mediated shiga like toxin - Verocytotoxin
- Pathogenicity similar to shigella
- I.P : 3-8 days
- Patient with Bloody Diarrhea, Abdominal cramps, and vomiting. Some with HUS
- most severe disease with HUS as common complication - E.Coli O157 : H7

Diagnosis :

(i) Gram smear : Pus cells, RBC +++
(ii) Culture : MacConkey medium
(iii) Prove EHEC :
- O157 H7 - Sorbitol MAC Test
  - can't breakdown sorbitol - Pale colonies
  - Rest E.Coli can breakdown sorbitol - Pink colonies
- Non O157 H7 : Cytotoxicity on vero cell lines

Treatment :

- Symptomatic. Antibiotics are C/I

✓ most other infections caused by E Coli are by Normal G.I flora which have acquired special virulence factors. They causes diseases when they enter a normally sterile extra intestinal site
- MCC of UTI in all age groups, males/females
  (i) Community acq. UTI
  (ii) Nosocomial UTI
- These are called as Uropathogenic E.Coli - virulence factor - 'P' pili
- MCC of intraabdominal abscess, Peritonitis
  MCC of Neonatal meningitis
- Nosocomial Pneumonia
- Wound infections / Burn infections etc.

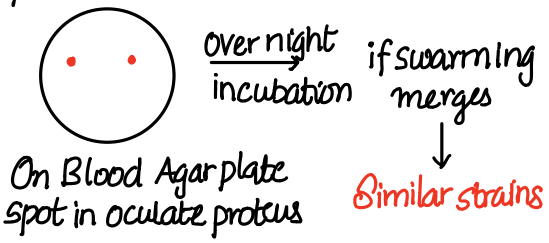
PROTEUS

✓ Motile, Non-capsulated
✓ Biochemical reaction : PPA +ve ie it convert Phenyl alanine → Phenyl pyruvic acid
✓ H_2S +ve on sugur fermentation
✓ Urease +ve

Culture :-
- On Blood agar : Swarming
- On MacConkey : NLF
- Fishy smell colonies

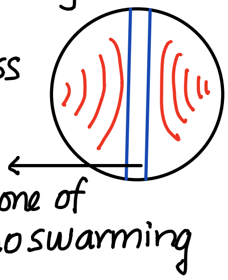
DIENE'S PHENOMENON

- Used for edidemiological typing of proteus strains



On Blood Agar plate spot in oculate proteus

- If differential strains - colonies are seperated by a zone of no swarming
- Proteus is also a normal gut flora. It can cause wound infection
  - (i) Intra abdominal abscess
  - (ii) UTI - Recurrent UTI due to proteus leads to Persistant Alkalinisation of urine leading to STURVITE STONE
  - (iii) Peritonitis
  - (iv) Nosocomial Pneumonia



Serratia Marcesans

- Produce red pigment PRODIGIOSIN
- Pseudohemoptysis
- Efficasy tester of the pore size of Std. bacterial filters

Enterobacteriaceae-II

SALMONELLA

✓ All are

1. Motile except S.gallinarum-pullorum
2. Non-capsulated except S. typhi, S. paratyphi C, S. dublin

✓ Two species : S. enterica & S. bongori → 6 subspecies (enterica, indica, arizonae)

↓

>3000 serotypes

(It's based on KAUFMANN & WHITE SCHEME :- O, H, Vi Ag are used)

✓ Salmonella typhi

⇒ S. enterica subsp. enterica serotype typhi 9,12 Vi : ⓓ → Flagelar Antigen

9,12 ↓ Oantigen; Vi ↓ Capsular Ag

✓ S. Paratyphi A ⇒ S. enterica subsp. enterica serotype paratyphi A

('O' antigen) 1, 2, 12 : a (flagellar antigen)

✓ S. Paratyphi B ⇒ S. enterica subsp. enterica serotype paratyphi B

('O' Antigen) 1, 4, 5, 12 ; b, 1, 2

b, 1, 2 → Flagellar Ag shows PHASE/ANTIGENIC VARIATION

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✓ All serotype causing enteric fever in India share the same 'O' 12 antigen in their cell wall. ∴ in a widal, 'O' antigen of S typhi detects antibodies against all 3 serotypes

BIOCHEMICAL REACTIONS:

- Aerogenic (except S. typhi)
- Non-lactose fermenters (NLF)
- H_2S +ve (except Paratyphi A, Cholerasuis, typhisuis)
- IMViC: − + − + (except S. typhi: − + − −)
- Urease −ve

ANTIGENS OF SALMONELLA

'H' antigen:

- Flagellar antigen
- Protein in nature
- Highly antigenic
- Heat & alcohol labile
- shows phase variations
- 'H' antibodies appears earliest infection & persist for several months.

'O' antigens:

- Somatic antigens
- polysaccharide
- lower antigenicity
- heat & alcohol stable
- 'O' antibodies follow appearance of 'H' Abs & disappear in few weeks

H Abs → Significant titre }
O Abs → Insignificant titre } Remote (or) Recent infection

H Abs → Significant titre }
O Abs → Significant titre } Recent or Active infection

Vi antigens

- capsular antigen
- polysaccharide
- least antigenic
- Heat labile & alcohol stable
- Present only in typhi, paratyphi C, Dublin also in some E. Coli & some Citrobacter
- Covers 'O' Ag & prevents agglutination with 'O' Antibody
- Vi Abs appear for a short time during covalescence
- Absence of Vi antibodies: Poor prognosis
- Persistence of Vi Abs: Carrier state

CULTIVATION

(i) On MacConkey medium: NLF Colonies
(ii) Transport: Cary Blair
(iii) Enrichment medium: Selenite F broth
Gram Negative broth
Tetrathionate broth
(iv) Selective media: DCA, XLD, SSA, HEA (Hekton Enteric Agar)
(v) Best selective media:
Wilson & Blair Bismuth medium
→ Black colonies due to H_2S production

DISEASES

1. Enteric fever / Typhoid
   - S. typhi (M.C cause in India)
   - S. paratyphoid A (Second M.C cause in India)
   - S. paratyphoid B
   - S. paratyphoid C
   - All four serotype are strict human adapted stains
2. Food poisoning or invasive Enteritis
   - All other serotypes (Non typhoid typhoidal Salmonella - GI commensals or pathogens of reptiles, birds, (or) mammals (other than human)
   - Zoonoses

TYPHOID / ENTERIC FEVER

- Strict human disease
- I.P: 1-2 weeks (3-21 days)
- Infective dose: 10^3 - 10^6 bacilli
- Clinical feature:
  Step ladder pyrexia, malaise, anorexia, vomiting
- On examination:
  Bradycardia (Faget sign → Fever c̄ bradycardia)
  Rose spots
  Hepatomegaly, splenomegaly
  Progress over 3-4 weeks

Notespaedia.com More than just notes...

COMPLICATION

1. Intestinal hemorrhage - MC } MC in
2. Intestinal perforation - next MC } 3rd wk
3. Meningitis, deafness, psychosis
4. Arthritis, periostitis, nephritis
5. Chole cystitis, visceral abscesses

DIAGNOSIS OF ENTERIC FEVER

A. Isolation by Culture
- Specimens collected

(i) Blood:

5-10 ml sterile blood in 50-100 ml of bile broth

↓

Subculture on MacConkey and blood Agar for 10 days

- Preffered: 5-10 ml sterile blood

↓

In Biphasic/Casteneda medium
(Brain-heart infusion agar with broth)

- Rate of positivity:
  - 1st week: >90%
  - 2nd week: ~ 75%
  - 3rd week: ~ 60%
  - 4th week: ~ 25%

(ii) Stool:
- Positive at end of 2nd week in 40-50% of cases
- Selective medium used for culture

(iii) Urine:
- Positive at end of 3rd week in 30-50% cases.

(iv) Bone marrow aspirate:
- 2 advantages:
  - (a) most sensitive at all stages
  - (b) Remains positive for ~ 5 days after start of treatment
- Similar to blood culture

SEROLOGY

1. Widal test:
- Tube agglutination test
- Significant titre of 'H' Ab + 'H' Antigen

↓

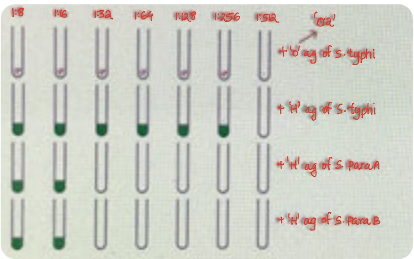
Fluffy/Wolly agglutination
- Done in Dreyer's tube (conical test tube)
- Significant titre of 'O' Ab + 'O' Ag

↓

Granular/Chalky agglutination
- Done in felix tube (round bottom test tube)

Interpretation of Widal test

- 4 sets of test tube with serial dilution of patient's serum
- 1st set: add 'O' Ag of S. typhi
- 2nd set: add 'H' Ag of S. typhi
- 3rd set: add 'H' Ag of S. paratyphi A
- 4th set: add 'H' Ag of S. paratyphi B



- significant titre of 'O' Abs: >100
- significant titre of 'H' Abs: >200

* Significant titre of 'O' Ab indicates recent infection

* Significant 'H' Ab titre indicates the serotype

2. Typhidot
- ELISA based method
- Immune-chromatographic test
- IgM & IgG Ab detected seperately
- No quantification

Serological tests are Neither sensitive nor specific

OTHER TESTS

B. Diazo test
- Biochemical test
- Detect phenolic compound in urine
- Good sensitivity & specificity only in 1st week

C. Antigen detection in serum
- ELISA/Latex agglutination/Co-agglutination

D. Probes/Nucleic acid Amplification test
- molecular method
- most sensitive techniques
- limited availability.

TREATMENT

- Empirical treatment
  - Ceftriaxone
  - Azithromycin
- Fully sensitive strains
  - Ciprofloxacin
  - Amoxicillin
  - Azithromycin
  - Cotrimoxazole
  - Chloramphenicol
- MDR strains: resistant to Amoxicillin, Cotrimoxazole, Chloramphenicol
- Treated using: 1) Ciprofloxacin
  2) Azithromycin
  3) Ceftriaxone (severe)
- NARST: Nalidixic acid resistant S typhi
  - also called FQ-resistant strains
  - Treatment: Azithromycin
    Cefriaxone

CARRIERS

- Based on site of persistance:
1. Fecal carriers:
   - more common
   - site of persistence: Gall bladder
   - seen in patient of billiary tract abnormalities
2. Urinary carriers:
   - site: Kidney
   - seen in: urinary tract abnormalities or schistosoma haematobium infection
- Based on duration:
1. Convalescent: upto 3 months
2. Temporary: 3 months - 1 year
3. Chronic: >1 year

Diagnosis:

(i) Screening: Vi agglutination test to detect Vi antibody

(ii) Confirmation: Culture

Specimen: Stool or duodenal aspirate
Urine

NON-TYPHOIDAL SALMONELLA

- causes zoonotic infections
- mode of infection:
  - a) Ingestion of raw milk/milk products/poultry/raw eggs
  - b) Handling pets (turtles/tortoise)
- I.P: 8-24 hrs (upto 48 hrs)
- Present with: fever, abdominal cramps, diarrhea, rarely blood in stools
- Self-limited
- Diagnosis: Stool Culture
- Most common serotype: S. typhimurium
- In young/old/immunodeficient/chronic hemolytic anemia, enteritis can progress to septicemia, complicated by Meningitis/Osteomyelitis/Nephritis
- Most common cause of osteomyelitis in sickle cell anemia: Salmonella
- Diagnosis: Blood Culture
  M.C serotype: S. cholerasuis, S. dublin
- Treatment: 3rd generation cephalosporins

YERSINIA PESTIS

- Non-motile
- Capsulated: 1 antigenic type
- Highly pleomorphic gram negative rods
- Grow at wide pH range: 5-9.6
- Grow at wide temp. ranges: 2°-45°C
  Optimum temperature: 27°C
- Cold enrichment: method of isolation of Yersinia or Listeria from a mixture of bacteria by Refrigeration

Biochemical Reaction:

- Anaerogenic
- NLF
- H_2S -ve
- IMViC -+--
- Urease -ve

Biotypes

biochemical reactions are used for typing
- 2 tests used: Glycerol fermentation
  Nitrate reduction
- 3 biotypes: Antiqua, Medievalis, Orientalis

CULTURE

- On MacConkey : NLF, Pale colonies
- On blood agar : dark brown colonies due to hemin deposition
- On ghee-broth : Stalactite growth
- Selective medium:
  Cefsulodin Irgasan Novobiocin Agar (CCIN Agar)

VIRULENCE FACTORS :

1. Polysaccharide capsule: Fraction-I Ag (F-I Ag)
2. Lipo polysaccharide
3. Protease :
   At 37°C → Fibrinolytic activity
   At 25-28°C → Coagulase activity
4. V and W proteins :
   Help in intracellular survival.

DIAGNOSIS OF PLAGUE

- Specimen : Bubo aspirate
  Sputum

(1) Wayson stain:
Simple stain, methylene blue
Safety pin / Bipolar staining

(2) Culture :
- Done only in Biosafety level III lab (BSL-III)

(3) F-I antigen detection by immunofluorescence

(4) Serology :
- Antibodies appear by 2nd week
- Detected by ELISA or IFAT

(5) Molecular methods
- most sensitive

PLAGUE

- Zoonoses
- Reservoir : Wild rats
- Vector : Rat flea
- Dangerous reservoir : Domestic rats

Types of plague :

1. Bubonic Plague : Most common
   - Acquired by bite of flea
   - I.P : 3-7 days
   - Present as : Painful regional lymphadenopathy (buboes)
   - 50% untreated cases : Spontaneous resolution
   - The remaining 50% → Septicemia
2. Pneumonic Plague :
   - Aerosol inhalation
   - I.P : 1-2 days
   - Hemorrhagic pneumonia with/without pleuritis/mediastinitis/meningitis
   - 80-90% untreated cases → Septicemia
3. Septicemic plague :
   - usually secondary to bubonic/pneumonic plague
   - Occasional primary manifestation
   - Severe endotoxemia ⇒ Gangrene of toes/fingers

TREATMENT OF PLAGUE

- Streptomycin or Gentamicin
- Levofloxacin
- Doxycycline

Pneumonic plague case ⇒
Isolation under negative pressure

Post-exposure prophylaxis :
DOC : Doxycycline

Pre-exposure prophylaxis :
Vaccine : Sokhey's modification of Haffkine vaccine
(Killed Y. pestis is used)

Vibrio AND Pseudomonas

VIBRIO

✓ Family: Vibrionacea (gram negative curved rods)
✓ Reservoir for all vibrio : Sea water
✓ General Charachteristics:
Morphologically : Gram negative, curved bacili
- Robert Koch called it as Commabacilli
- Monotrichous
- Darting/Shooting star mortality
- facultative anaerobes except V. cholerae - Poor growth under anaerobic condition
- Alkaliphiles (optimum pH → 8.2 – 8.4).
- Require salt to grow: Halophiles (Optimum salt conc → 0.5 - 1%)

V. Parahemolyticus	→ salt conc (2-4%)
>6% salt	→ Growth is inhibited
Upto 8% salt	→ survived by V. parahemolyticus
Upto 10% salt	→ Survived by V. alginolyticus
Non halophic	→ V. cholerae & V. mimics

Biochemical Reaction

- Catalase positive
- Oxidase positive
- Nitrate reductase positive
- String test positive
  Suspension of Vibrio + bile salt
  ⇓
  String formation
- Two special biochemical reactions for V. cholerae
  1. Cholera Red Reaction:
     Reduce nitrate → Nitrite
     Convert tryptophan → Indole

     Nitrite + Indole → Nitrosoindole
     +
     Red colour ← H_2SO_4
  2. Gelatin stab culture
     Napiform/infundibuform liquefaction

Culture

✓ Simple media
MacConkey medium
(i) Non Lactose fermenting colonies
(ii) After few days Late Lactose ferme.

✓ Transport medium:
V.R Medium, Autoclaved Sea water, Cary blair medium

✓ Enrichment medium:
- can be used as transport medium
- Alkaline Peptone water
- Monsur's taurocholate tellurite peptone water

✓ Selective medium
- Bile salt agar
  Monsaur's GTTA (Gelatin Trypticase Taurocholate Tellurite Agar)

✓ Selective & Differential medium
- TCBS
  (a) Surcose fermenting Vibrio (Yellow)
  → V. cholerae
  (b) Non surcose fermenting Vibrio (pale) → V. parahemolyticus

CLASSIFICATION OF VIBRIO

- By Gardner & Venketaraman

Based on the H antigen of V. Cholerae

- +ve → Group A
  - V. cholera
    - Sakazaki serotyping (Based on somatic Ag (O Antigen) that is part of LPS
      - O_1 to O_{200} Serogroups
- -ve → Group B
  - All othe species

- 2 serogroups that produce cholera toxin causing cholera disease : O_1 & O_{139}
- O_2-O_{200} (Other than O_1 & O_{139}) → Non cholera vibrios
- O_2-O_{200} (Other than O_1) including O139 → Non agglutinable vibrios
  - Do not agglutinate with O_1 antiserum

V. CHOLERAE O1

V. cholerae

Biotypes

- El Tor
  - Ogawa (A, B)
  - Inaba (A, C)
  - Hikojima (A, B, C)
- Classical biotypes
  - Ogawa (A, B)
  - Inaba (A, C)
  - Hikojima (A, B, C)

Based on A, B, C Antigens - Serotyping

		El Tor	Classical
✓ Sheep RBC hemolysis	:	+	−
✓ Chick RBC agglutination	:	+	−
✓ VP test	:	+	−
✓ Susceptible to Polymyxin	:	Resistance	Sensitive
✓ Susceptible to phage	:	5	IV
✓ Hardier (Survives longer in the Envionment)	:	Hardier	Less hardier
✓ Disease	:	Milder	More sever
✓ Mortality	:	Less	More
✓ Secondary attack rate	:	Less	More
✓ Subclinical cases	:	More	Less
✓ Carriers	:	More	Less

V. Cholerae O139

- First isolated in Chennai in 1992
- Also known as Bengal strain

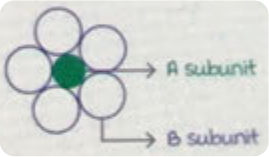
* Like V. cholerae O1
  Cholera toxin + disease similar

* Unlike V. cholerae O1
  - Capsulated
  - agglutinate with O139 antisera
  - Presently reported from India & Bangladesh.

- No cross immunity b/w O_1 & O_{139}
- Pandemics
  - 7 in the last 200 yrs
  - First six pandemics → Indian subcontinent
    V. cholerae O1 (Classical)
  - Seventh/present → Indonesia
    V. cholerae O1 (El Tor)

PATHOGENICITY FACTOR

1. Sheathed flagellum
2. Toxin coregulated pili
3. Phage mediated → CTX ϕ
   AB5 Subunit toxin (84,000 Da)



Receptor : GM_1 ganglioside
Site of action : by ↑ cAMP
A subunit → $A_1 + A_2$
⇓
ADP Ribosylation of α subunit of of G stimulatory protein (GS_α)
↓
Persistent activation of adenyl cyclase
↓
↑ cAMP
↓
Persistent activation of cAMP dependent protein kinase
↓
Phosphorylation of CFTR (Cystic Fibrosis Transmembrane Regulatory protein)
↓
Secretion of Ions into intestinal lumen

4. Neuraminidase : generate receptors for Cholera toxin

* LPS / Endotoxin has no role in Cholera

CHOLERA

- strict human disease
- Reservoir : Human
- I.P : 2 to 5 days
- Infective dose : $>10^6$ bacilli taken with water
  10^3 when taken with food

Vibrios are highly susceptible/sensitive to acid pH of stomach
Food reduces gastric pH → Thus ↑ infectivity

- Risk factors :
  - Antacids / PPI, H_2 blocker
  - Hypo/achlorhydria
  - gastrectomy
- O blood group patient develop severe disease

Clinical features :
- Effortless vomiting
- Watery diarrhea
  (Rice water stool - mucous flakes in stools)
- Fever in children only

- Endemic : mainly children effected
- Epidemic : all ages are effected.

- 1 episode : protective immunity last for only 6 to 12 months

DIAGNOSIS AND TREATMENT OF CHOLERA

Diagnosis :

1. Wet mount : Fish in stream appearance
2. Gram stain : Gram negative, curved rods
3. Selective medium : Isolation of bacteria
4. Toxigenicity tests :
   (i) Y_1 adrenal tumor cell assay
   (ii) Chinese haster ovary assay

Treatment :

→ Rehydration
→ Antibiotics reduce the duration/ severity of diseases
DOXYCYCLINE : 300mg single dose
CIPROFLOXACIN : for 3 days
AZITHROMYCIN : 1g single dose
– Chemoprophylaxis not required even in household contacts

GROUP B VIBRIOS

- V. parahemolyticus
  - Peritrichous : flagellae on solid media
    Monotrichous : flagellae on liquid media
  - Capsulated
  - 2 to 4% salt to grow
  - Tolerate upto 8% salts
  - Swarming on Blood agar

- TCBS medium: Non sucrose fermenting
- Disease caused by ingestion of contaminated sea food.

↓

Invasive Diarrhea
(may be bloody)

- if required Doxycycline can be given

Kanagawa phenomenon:
- On Wagatsuma agar
  (Blood agar prepared using human blood with 2-4% salt)
- Pathogenic strain: Hemolytic
- Non pathogenic strain: Non-hemolytic

- Vibrio vulnificus:
  - Also called as L+ Vibrio
    (Only lactose fermenting species of vibrio)
  - Disease caused:
    1. Following intake of contaminated Sea food

Healthy
↓
Toxin mediated diarrhea

Patients with cirrhosis/ Iron overload stage/ Infected with HIV
↓
Septicemia with myonecrosis

2. Cellulitis of wounds acquired in sea water.

V. Alginolyticus
- Grow upto → 10% salt
- Swarming on blood agar
- Disease:
  - * eye/ear infections in sea water swimmers
  - * cellulitis of wounds in sea water

PSEUDOMONAS

Psedomonas aeruginosa
- Gram negative bacillus
- Monotrichous
- Non capsulated
- Produces slime
- Strict aerobe
- Can grow upto 42°C

Biochemical Reactions

Catalase +ve
Oxidase +ve
Nitrate reduction +ve
Oxidative utilization of sugars - Nonfermentor

Culture:
- Non fastidious → grow on simple media
- On nutrient agar:
  Produces pigment: Pyocyanin (100%) - Bluegreen
  Pyoverdin (+/-) - Yellowgreen
  Pyorubin
- On blood agar: mucoid, β-hemolytic colonies
- Selective media: Cetrimide agar

Virulence factors of Pseudomonas:
(i) Lipopolysaccharide
(ii) Adhesion: Slime
Common fimbrae: Type I & Type IV
Adhesin proteins
(iii) Tissue invasion:
- Exotoxin A → ADP-Ribosylation of EF-2 (Elongation factor)
- Hemolysin → Phospholipase
- Exoenzymes S, T, U, V

(iv) Quorum Sensing:
- Cell to cell communication for coordinated expression of virulence factors.
- Pseudomonas secrete auto-inducers

↓

at lower amount of auto inducers - no virulence factor produced.

↓

At threshold level of auto inducers, enough Virulence factors are to overcome host immune responce

INFECTIONS BY PSEUDOMONAS

1. Community infections
- ✓ MCC of corneal ulcers in contact lens users
- ✓ Malignant otitis in diabetes
- ✓ Swimmer's ear
- ✓ Green nail syndrome
- ✓ Hot-tub folliculitis / Jaccuzzi syndrome
- ✓ Shangai fever → Enteric illness

2. Nosocomial infections:
- ✓ MCC of burn infections
- ✓ MCC of ventilator associated pneumonia
- ✓ Septicemia in immunodeficient (neutropenia)

  Bullous skin lesion or ecthyma gangrenosum
- ✓ Post cathorization UTI
- ✓ Post lumbar puncture meningitis

BURKHOLDERIA MALLEI:

- Strict equine reservoir
- Causes Glanders/Farcy buds/pipes of horses
- Mode of infection:
  Aerosis or percutaneous
- Disease caused: Pneumonia
  Skin lesions
- Complication: Septicemia

Exceptions:
- not an environmental saprophyte
- non motile
- Oxidase Negative

Treatment same as Pseudmallei

BURKHOLDERIA CEPACIA

- Soil saprophyte
- Causes: • Pulmonary infection in cystic fibrosis
  • Chronic granulomatous disease

TREATMENT OF PSEUDOMONAS

* multidrug resistant due to:
  (i) R-plasmid encoding hydrolytic enzymes of efflux pumps.
  (ii) Lack of porins on outer membrane

* inherently resistant to pencillins, cephalosporins (1 & 2 generation)

* Carbenicillin, ticarcillin, Azlocillin, Monobactam can be used.

BURKHOLDERIA

- 3 species: B. pseudomallei
  B. mallei
  B. cepacia

BURKHOLDERIA PSEUDOMALLEI:

- also called as Whitmore bacillus
- category B Bioterrorism agent
- BSL-III pathogen
- Bipolar staining - Safety pin appearance
- Present in soil & water of South-East asian countries
  - Saprophytes

Mode of infection:
(i) Aerosol ⇒ Causes Pneumonia
(ii) Percutaneous ⇒ Skin lesions associated with Lymphangitis & Lymphadenitis

Complication: Septicemia

Diagnosis:
- Culture on Ashdown's medium

Treatment:
Induction phase:
Ceftazidime/Carbepenam × 2 weeks
Maintainance phase:
Doxycycline × 2-3 months

Diseases: Meliodosis / Vietnamese time bomb
(∵ it can cause latent infection)

Neisseria

- ✓ Gram negative diplococci
- ✓ Non-motile
- ✓ Non-sporing
- ✓ Strict aerobes
  (except Gonococcus → Facultative anaerobe)

Commensal vs Pathogenic species

- Commensals → Not capnophilic
- Commensals can grow upto 22°C
  (Pathogenic species : 35-37°C)
- Commensals : Nutrionally non fastidious
- Commensals : Do not grow in selective media

Culture

Pathogenic species : Nutrionally fastidious
⇓
Require blood, hemin, special animal products for it's growth

- Blood Agar :
- Chocolate Agar :
- Selective media :

(i) Thayer Martin medium (Vancomycin + Colistin + Nystatin)
(ii) Modified Thayer martin/ New york city medium
↓
Vancomycin + Colistin + Nystatin + Trimethoprim
(iii) Martin Lewis Medium

- Transport medium :

(i) Stuarts medium } semisolid
(ii) Amie's medium } medium
(iii) Charcoal + Stuart's medium
better than Stuart's medium

Non pathogenic species:

(Normal upper respiratory tract flora)

- N. mucosa
- N. sicca
- N. lactamica
- N. flavescens } produce
- N. subflava } Pigmented colonies

Biochemicals

Catalase positive
Oxidase positive
Utilise sugars : all species utilise Glucose
Only N. sicca → Glucose
Only N. lactamica ⟶ Lactose
All utilise Maltose except Gonococcus

NEISSERIA MENINGITIDIS

- lens shaped
- Strict aerobe
- Capnophillic
- Capsulated
- Sugurs utilized → Gluecose & Maltose

✓ Typing based on Capsular Ag :

- 13 capsular serotypes (A, B, C, D....)
- 90% meningococcal disease is due to A, B, C serotypes
- Serotype A :
  - Associated with epidermis
  - MC cause of Meningococcal disease in India
- Serotype B
  - Both epidemic & outbreak
- Serotype C
  - Associated with outbreaks
- Serotype X, Y, W-135
  - Sporadic cases of meningococcal disease.

✓ Other typing methods

- On the basis of membrane probeins
  Por B : serotypes
  Por A : subserotypes
- Multilocus enzyme electrophoresis
- Multilocus sequence typing (MLST)

VIRULENCE FACTORS

1. Lipo oligo saccharide / Endotoxin:
   Amount of endotoxin → 50-100 times more compared to other gram -ve organisms.
2. Polysaccharide capsule
3. Pili / Fimbriae
4. Outer membrane proteins → Por B & A
5. Iron binding proteins
6. IgA1 protease

RISK FACTOR FOR DISEASE

1. Age
   Peak → 1st year
   Peak → 15-25 yrs
2. Overcrowding:
   Hajj piligrims, dormotories, refugee camp
3. Winter or dry season
4. Traveller to Meningitis belt
5. Smokers
6. Terminal complement factor, properdin, factor D deficiency
   ↓
   500-600 times more reported
7. Infection with Mycoplasma
8. Recent viral infection
9. Hypogammaglobinemia
10. Splenectamized patients.

EPIDEMIOLOGY & TRANSMISSION

Epidemiology:

- menigococcal disease reported World wide
- meningitis belt → Sub saharan Africa (Medittaranean belt)
- Reservoir: Humans
- Site for carriage: Nasopharynx
- Inter epidemic period:
  Carriage rate: 5-30%.
- During epidemic period:
  Carriage rate: 50-70%.

Transmission:

Air borne droplet of nasopharyngeal secretion of the disease

→ Hematogenous

→ Along the perineural sheath of Olfactory nerve

MENINGOCOCCAL DISEASE

→ Meningitis: MC presentantion (30-50%)

→ Menigococcaemia (20%)

→ Both (40%)

- **Meningitis:**
  - fever, headache, neck rigidity
  - Petechial / purpuric rash (60-75%)
    - Extensive purpura known as P. Purpura fulminans

- **Menigococcaemia:**
  - fever, headache, abdominal pain, chills
  - Hemorrhagic skin rash
  - Poor peripheral perfusion leading to Cold clammy limbs, tachycardia
  - Severe disease:
    DIC, Shock, Multi organ failure known as Waterhouse friedrichson syndrome
  - Mortality rate: 40%.

COMPLICATIONS

- MC: Scarring of the necrotic skin lesions - 10%
- Neurological & Psychological complication.
  (Neurological = Deafness)
- Post menigococcal Reactive Disease
  - Rare complication of menigococcal disease
  - Immune complex mediated
  - Presentation:
    Fever with
    → Arthritis (MC)/
    → maculopapular/vasculitis rash
    → Iritis/
    → Pericarditis or polyserositis
  - usually undergo spontaneous resolution

TREATMENT & PROPHYLAXIS

Treatment:

- DOC: 3^0 Cephalosporins
  ↓
  IV Ceftriaxone/Cefotaxim x 1 week
  (Blood/CSF becomes negative within 24hrs)
- Incase of allergy to Cephalosporins
  → Chloramphenicol

Chemoprophylaxis:

- Recommended for:
  - Intimate & (or) house hold contacts of the index case
  - Health care workers exposed to resp. secretions
- Adults: Ciprofloxacin → Single dose/
  Ceftriaxone → Single dose/
  Rifampicin → 4 doses in 2days
- Pregnant women: Ceftriaxone
- Children: Rifampicin x 2days/
  Ceftriaxone

DIAGNOSIS OF MENINGOCOCCAL DIS.

For meningitis:

⇒ Specimen: CSF Sediment
↓
(i) Gram staining:
Gram -ve lens shaped diplococci
(ii) Culture on blood Agar/Chocolate Agar
⇓
moist glistening colonies
(iii) PCR

⇒ Specimen: Supernatant
⇓
Slide Agglutination/Latex agglutination for capsular Antigen

⇒ Specimen: Skin biopsy (of the rash)
- Gram staining
- Culture
- PCR

For meningococcaemia:
Blood culture in Brain Heart Infusion Broth
↓
Subculture in Blood Agar & Chocolate Agar

For carrier:
⇒ Specimen: Nasopharyngeal swabs
↓
Cultured in the selective medium

Vaccine:

- Polysaccharide capsular vaccine
  - Available as monovalent, bivalent, quadrivalent vaccine
  - Again the capsular serotype A,C,W,Y
  - Conjugated with Diphtheria toxoid/Tetanus toxoid/CRM-197

$$\underbrace{\text{Diphtheria toxoid/Tetanus toxoid/CRM-197}}_{\text{Carrier protein}}$$

Carrier protein
⇓
Enhance the immunogenicity

- Outer membrane proteins based vaccines
  - For Serotype B (as their capsular Ag show molecular mimicry)
  - Men B-F Hbp → 3dose schedule
    Men B4C Bexsero → 2dose schedule

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NEISSERIA GONORRHOEAE / GONOCOCCI

- ✓ Gram negative, bean / kidney shaped diplococci
- ✓ Non-motile
- ✓ Non-sporing
- ✓ Non-capsulated
- ✓ Facultative anaerobes, Capnophillic
- ✓ 95% of gonococci strains → Small cryptic plasmid of unknown function present.

Typing:

1) Based on → Por protein

Por B1A | Por B1B

Por B1A → more propensity to cause disseminated infection

2) Auxotyping → utilizing certain substrate (Arginine, Uracil, Methionine etc)

AHU Auxotype → Slower growth
→ more tendency to cause disseminated dis

AHU → Arginine, Hypoxanthine, Uracil

3) Opa gene typing
4) MLST & DNA sequencing

Biochemicals

- Catalase +ve
- Oxidase +ve
- Utilise only glucose

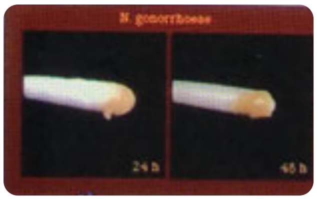
Cultivation

- On chocolate / blood agar
↓
round convex or umbonate colonies

Type-1, Type 2 | Type 3, Type 4

Type-1, Type 2:
- Brown, small
- shows autoagglutination
- formed by Virulent strains (piliated)
↓
P^+ & P^{++}

Type 3, Type 4:
- white, large
- forms smooth suspension
- avirulent strains (Non piliated)
↓
P^-



Virulence factors

1. Gonococcal lipo oligo saccharide show molecular mimicry:
Resembles human glycosphingolipids
↓
   - Evasion of host immune responce
   - Protects from complement mediated lysis.
2. Pili (Fimbriae) → Help in adhesion
→ Antigenic variation
3. Por protein (Protein I) → • Forms channel for the uptake of nutrients
   - Inhibits fusion with Lysosome
   - Anticomplementary
4. Opa protein (Protein II) → • Adhesion among gonococci & host cell.
   - Invasion into host cells
5. Rmp (Protein III) → Reduction modifiable protein
Associates with the Por protein
6. Ig A, Protease →
Also produced by meningococcus, Pneumococcus, H. influenza
7. Transferring binding protein (Tbp 1 & 2)
& Lactoferrin binding protein
This will help in Iron scavenging

Antigenic variations:

- Have multiple genes → Interchange for expression
- Seen in Pilin, Opa, Lipo oligosaccharide

GONORRHEA

- strictly human disease
- sexual transmission
- Reservoir → Asymptomatic carrier
  - ↓ Urethra
  - ↓ Convex
- I.P: 2-8 days

Gonorrhea in males:

- Primary site of Infection: Urethra → Prostate → Epididymis
- MC presentation: Urethritis
- Prostatis, Epididymitis
- Usually symptomatic

⇓

Initially: mucoid urethral discharge → Purulent + dysuria

Gonorrhea in females:

- Primary site of infection: Cervix
  ⇓
  Urethra & Bartholin glands
  (Urethritis + Bartholinitis)
- Usually asymptomatic (50-80%)
- Complaints of purulent vaginal discharge dysuria & Urethral discharge
- Can spread to uterus ⇒ Endometritis, Salpinigitis
- PID, Fitz-Hugh Curtiz Syndrome (Perihepatitis)
- Spread to perineal tissues → multiple perineal abscess/sinuses
  ↓
  WATER CAN PERINEUM
- Anal sex: Proctitis (tenesmus, rectal discharge)
- Orogenital exposure: Pharyngitis

Ocular Gonorrhea:

- ↓ Perinatal transmission
  ⇓
  Acute conjuctivitis
- ↓ Autoinoculation

GONOCOCCAL BACTREMIA

- manifest even without urogenital symptoms
- MC: Por BIA & AHU auxotypes
- Risk factors:
  Menustruation & Terminal complement deficiencies.
- 2/3rd of the infected ⇒ females
- Disseminated Gonococcal infection:

(i) MC: Skin
  - ↓ Papular/Pustular lesions + Hemorrhagic component
  - ↓ Nodules, urticaria, Erythema multiforme

(ii) Suppurative arthritis:
  MC: Knee

(iii) Tenosynovitis

(iv) Osteomyelitis → Small joints of hand

(v) Meningitis/Endocarditis ⇒ Rare

DIAGNOSIS

- Specimens: Pus & secretions from the urethra, cervix, rectum, conjuctiva, throat or synovial fluid

(i) Gram staining: Gram negative diplococci

(ii) Immunofluorescence:
  Antigen detection

(iii) Culture: Blood Agar/Chocolate Agar
  Selective media:
  Thayer martin/Modified Thayer martin

(iv) NAAT: Most sensitive

Serology is not useful as gonococcal ANITIGENIC HETEROGENICITY

RESISTANCE TO ANTIBIOTICS

Due to chromosal mutations / R plasmid

- Pencillin Resistance
  - R plasmid mediated → Pencillinase producing N. gonorrhoea (PPNG)
  - Gene Mutations → Pencillin Binding protein
- Tetracycline Resistance ⇓ R plasmid mediated → Tet M gene → Prevent binding to ribosome (often associated with PPNG)
- Quinalone Resistance ⇓ 20-30% strains → Gene mutation in gry A + par C genes

TREATMENT OF GONNORRHOEA

1. Uncomplicated Gonococcal infection ⇒ Ceftriaxone 250 mg in single dose
   ↓
   Alternative:
   - Cefoxitin 2g in IM single dose
   - Ceftizoxime 500 mg in single dose
   - Cefotaxim 500mg in single dose

   +
   AZITHROMYCIN 1g (Chlamydia)
2. MDR Strains: Septinomycin 2g IM
3. Disseminated Gonococcal infection
   - Arthritis, Tenosynovitis, skin lesion → Ceftriaxone IM or IV × 2days ⇓ Cefixime × 5 days
   - Gonococcal meningitis → Ceftriaxone IV × 2 weeks
   - Gonococcal endocarditis → Ceftriaxone IV × 4 weeks
4. Ophthalmia neonatorum
   Single dose of IV ceftriaxone (25-50 mg/kg)
   Prophylaxis: Topical 1% silver nitrate solution
   Erythromycin / Tetracycline ointment at the time of delivery

Treponema

Pathogenic genera:

- They belong to the order Spirochaetales:
  - Family : Spirochaetaceae : This contain treponema & Borrelia
  - Family : Leptospiraceae : contain Leptospira
  - Family : Brachispiraceae : contain Brachyspira

TREPONEMA

✓ Contain 2 species which are pathogenic to human

1. T. pallidium : Variety pallidium - Veneral syphilis
   Variety endemicum - Endemic syphilis
   Variety pertenuae - YAWS/FRAMBOESIA
2. T. carateum : causes PINTA

✓ All the species are morphologically & antigenically similar

TREPONEMA PALLIDIUM VAR. PALLIDIUM

✓ first discovered by Shauddin & Hoffman

✓ Generation time - 30-33 hrs

✓ killed at 4°C in 1-3 days, Hence we can refrigerate blood for 4 days to prevent transfusion syphilis.

Non-pathogenic treponemes

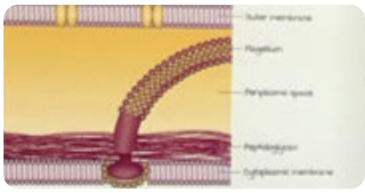
- They are normal oral & genito urinary flora

Eg: T. denticola
T. macrodenticum
T. phagedenis : Reiter's strain
T. refrigens : Noguchi's strain

These are cultivable on cell free medium known as Noguchi's

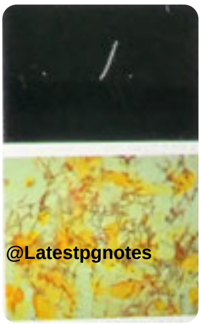
MORPHOLOGY

- it's a gram negative spiral organism
- 10-14 μ long, 0.18 μ broad
- It has tapering at ends & spirals are regullarly spaced. (Borrelia - irregularly spaced)
- They have ENDOFLAGELLA present at periplasmic space.
- 3-4 endoflagella are present
- It has CORK-SCREW motility



DEMONSTRATION

1. On microscope : Negative ink
2. Dark field microscope
   Phase contrast microscope
3. Prolonged Giemsa :
   Treponemes appear Rose pink
4. Silver impregnation
   - Fontana's stain for films
   - LEVADITI for tissue sections



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CULTURE:

- Obligate intracellular (∵ It's genome is highly restricted ∴ contain less synthetic enzyme)
  ∴ can't be cultivated on a cell free medium.
- 1st strain cultivated on Rabbit testis - Nichol's stain
- Limited replication in NELSON'S, EAGLE & MacCoy cell culture medium

SYPHILIS

✓ Also called as French disease
✓ it's a S.T.D (strict human pathogen)
✓ I.P : 9-90 days

SYPHILIS - PRIMARY

- Called as HARD/HUTARIAN CHANCRE
- Painless papule → ulcer with firm base and raised regular edges
  i.e Painless non tender indurated ulcer
- Regional Lymphadenopathy also present (firm & non-tender)
- Spontaneous resolution in 4-8 wks

SYPHILIS-SECONDARY

- This appear 2-8 wks after the appearance of Primary Chancre
- Generalised Lymphadenopathy
- Macular/Papular/Pustular rash
  - start from trunk & proximal extremities
- Palms & soles are effected
- Condyloma lata
  - In Axilla, Anogenital & oral mucosa
  - MOST INFECTIOUS LESION
- Mucus patches : Silvery grey lesions
- Symptoms of Hepatitis, Nephrotic syndrome, Meningitidis, Arthritis, Periostitis, Chorio retinitis, Uveitis, Iritis. So 2° symphilis is called as
  OLD GREAT IMITATOR
  (LATEST GREAT IMITATOR - Lyme disease)
- Resolves spontaneously in few months

SYPHILIS - LATENCY

- Serology +ve, but no symptoms
- Early latency: <1 yr of 1° chancre
  Relapse of 2° syphilis
- Late latency: >1 yrs of 1° chancre

ARP : ARGYLL ROBERTSON PUPILS

SYPHILIS - TERITIARY

- 40% untreated goes to teritiary stage.
  It appears 5-30 yrs after 1° infection.

(1) Gummas (Granuloma) -
- MC manifestation of 3° syphilis
- MC site :
  Skin → punched out ulcers
  Bones → fractures
  Liver

(2) CNS : Neurosyphilis
is due to

↓ Meningo vascular
(due to End arteritis obliterans)
- EARLIER to appear
↓
Symptoms : Hemiparesis
Hemiplagia, Aphasia,
blindness

↓ Parenchymatous
(due to destruction of neuron)
- LATE to appear

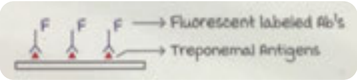
↓ Brain
↓
Parasesis of insane
P Personality d/o
A Affect d/o
R Reflexes (↑)
E Eye - ARP
S Sensorium
I Intellct
S Speech slurring

↓ Spinal Cord
↓
Tabes dorsalis
- Bladder incontinence
- Paresthesia
- Wide gait

(3) CVS
End arteritis obliterans of vasa vasorum of
Aorta
↓
- Aortitis
- Saccular Aortic Aneurysms
- Aortic regurgitation

DIAGNOSIS

1. Gold standard test: Rabbit infectivity test
2. Microscopic methods:
   - in early syphilis
3. Antigen detection
   - in early syphilis
   - Done by Direct fluorescent Ab testing. Flourescene isothiocyanate is used for conjugation



4. Serology : MC used

A. Non-treponemal/standard test
- Ag used : CARDIOLIPIN
  it's an alcoholic extract of ox heart
- Lecithin & Cholesterol are added to ↑ the antigenicity of cardiolipin
- The whole complex is known as the LIPOIDAL AG.
- Ab detected : Nonspecific Ab k/a Reagin Ab
  It's formed as a result of antigens which are released from damaged syphilitic tissue.

B. Treponemal test (T.T)
- Ag used: T. pallidium
- Ab detecting: Specific antitreponemal Ab

Types of Non-Treponemal test

1. Wasserman's Test
   - Type of complement fixation test
   - First test used to diagnose syphilis
2. KAHN Test
   - Type of tube flocculation test
   - It's a precipitation test
3. VDRL Test
   - Slide flocculation test
   - Disadvantage:
     - Require microscope
     - Inactivation of serum required
     - It's an unstable Antigen
   - Easy to use
4. Rapid Plasma Reagin (RPR) test
   - All the disadvantage of VDRL has been overcome by RPR.
   - Lipoidal Ag coated on carbon particles
5. TRUS (Toludine Red Unheated Test)
   - Ag coated on Toluidine Red Particles

Major disadvantage of NTT's are biological false positive, 1% of total population will give biological false positive

Types of Treponemal test

1. Treponema pallidium immobilization test:
   Live treponemes + Serum + complement
   ↓
   Immobilisation of treponemes are seen under dark field microscope
   - It's a complement based test
   - Not used now.
2. FTA -Abs:
   - Fluorescent Treponema Ab Absorbent test
   - It's Indirect fluorescent Ab test.
3. TPHA
   - Also called as MHA-TP - Micro hemagglutination if we do it in micro titre plates.
   - Treponemal Ag coated onto sheep RBC's
     ↓
     mix with pt's serum → Agglutination
4. TPPA
   - Treponema palidium Particle Agglutination
   - Trep Ag coated onto gelatin particles.
5. INDIRECT ELISA

False positive Treponemal test: Lyme's disease

Causes of biological false positive:

A. Acute (lasting <6 months)
- Measles, mumps, malaria, chickenpox viral hepatitis, Pneumococcal pneumonia, recent immunisation, Relapsing fever, Acute HIV infection
- Pregnancy & infectious mononucleosis
- Ab titre in biological false positive - ≤8

B. Chronic (>6 months)
- SLE, Lepromatous leprosy, collagen d/o, Nacrotics, Malignancies - Myelomas, Autoimmune disorder

Important points regarding Serological Diagnosis:

- It become positive, 10-14 days after the appearance of chancre.
- First test to become positive - FTA-Abs
- For Δ of syphilis - 2-step approach

  (a) Screening - with NTT (VDRL/RPR)
  (b) Confirmation - with TT (TPPA/TPHA)
  - follow up done with a NTT - after 6 month. Look for a 4fold fall in Ab titre
  - Treponemal tests remain positive years after successful treatment.

- Sensitivity

	1°	2°	3°
TT	85-90%	100%	~95%
NTT	80-85%	100%	70-73%

- Most sensitive: FTA-Abs
- Specificity:

TT - 98-99%
NTT - 97-99%

MOST SPECIFIC : TPPA > TPHA

DIAGNOSIS OF NEUROSYPHILIS

- Detect Antibodies in the pt's CSF.

VDRL-CSF : most specific
FTA-Abs-CSF : most sensitive

- Molecular methods : PCR or nucleic acid probes

NON-VENERAL TREPANOMATOSIS

- Also known as Endemic trepanomatosis
- Transmitted by contact of mucus membrane/precutaneous exposure to lesion of the disease
- I.P : 5-10 days
- No congenital transmission
- CVS/CNS involvement - Very rare

TREATMENT OF SYPHILIS

- DOC : Pencillins
- Early syphilis : Single dose of Benzathine Pencillin
- Late syphilis/Syphilis of unknown duration : 3 dose of Benzathine pencillin.
- Pencillin allergic : Doxycycline x 2-4 wks
- Pregnant female:
  Pencillin (Benzathine) only → if allergic to pencillin → Desensitisation required
- Neurosyphilis:
  Aqueous crystaline pencilin x 2 wks
  If allergic → desensitisation

NAME	1°	2°	3°
1. Bejel	• Small chancre in mouth • Usually goes unnoticed	• Rash • Mucus patches	• Gummas • CNS involvement - Rare
2. Yaws	• Ulcerating extra-genital chancre called as Mother yaws/Framboesioma	• Rash • Mucus patches • Mother yaws like skin lesion. It can involve the underlying	• Gummas known as Gangosa (destroy nose cartilage)
3. Pinta (mildest)	• Lichenoid/Psoriaform extra genital chancre	• Hypo/Hyper pigmented skin lesion	• Rare

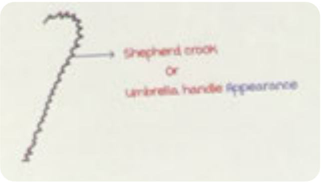
Treatment : Single dose of Azithromycin or Benzathine Pencillin

- Only Yaws is reported from india among the three, but it has been eliminated in India in 2006.

Leptospira AND Borrelia

LEPTOSPIRA

✓ It has 10 pathogenic species : L. interrogans
L. noguchi
↓
further divided into >26 groups → >250 serotypes/serovars



Morphology :

- Gram negative spiral
- 6-20 μ long
- 0.01 μ broad
- Endoflagellate

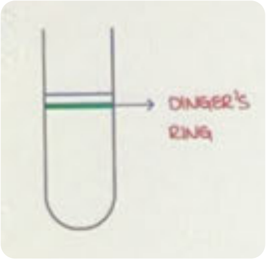
Microscopic Demonstration :

- Dark field microscopy
- Negative staining
- Silver impregnation

CULTIVATION

1. Serum media :
   a) EMJH
   b) Korthoff's
   c) Fletcher's
   d) Stewart's

These media → either liquid/semisolid
↓
Leptospira grows just beneath the surface of medium
↓
DINGER'S RING



- It takes 3wks - 3months for growth

2. Chorio-allantoic membrane of hen's egg
3. Intraperitoneal Guinea pig inoculation

EPIDEMIOLOGY

- most important reservoir → Rats
- other reservoirs :
  wild animals & Domestic animals
  (Dogs, cows, pigs, sheep)
- Asymptomatic colonization of Collecting tubule of Kidney
- MODE OF INFECTION
  Percutaneous/mucus membrane exposure to urine contaminated water.
- DISEASE COURSE :
  40% : Asymptomatic
  60% : → 90-95% : ANICTERIC FEBRILE ILLNES
  → 5-10% : WEIL'S DISEASE or ICTERO-HEMORRHAGIC FEVER

LEPTOSPIROSIS is one of the MC zoonotic infection in the world.

WEIL'S DISEASE

- I.P : 2-10 days (1-2 weeks)
- This is a biphasic disease

Stage of Bacteremia:

- Charachterised by Nonspecific symptoms
  Fever, Myalgia, Arthralgia,
  Conjuctival suffusion, Rashes.
- Usually seen in 1st week.

Immunological stage (In 2nd week)

Jaundice
+
Acute Renal failure
+
Hemorrhages

- Mortality → 10-15%

Diagnosis:

1. Blood → Positive only in 1st week
   - Dark field microscopy
   - Cultivation
   - Animal inoculation
   - NAAT's
2. Urine → Positive in second week
   - DFM
   - Cultivation
   - Animal pathogenicity
   - NAAT's
3. Serology :→ Positive in Second week.

(i) Genus specific tests
↓
by CFT/IFAT
(Complement fixation test/
Indirect fluorescence Ab test)
↓
PATOC 1 strain of Leptospira
biflexa

(ii) Serotype specific Antibody
↓
By Microscopic Agglutination
Test (most specific)
Done only in Reference Laboratory.

TREATMENT

- mild disease
  Oral → Doxycycline, Ampicillin, Amoxicillin } For 7 days
- moderate / severe disease
  IV Pencillin/Ceftriaxone/Cefotaxime/
  Doxycycline
- Prophylaxis
  Given for Sewer workers
  Abbatoir workers
  Rice field farmers
  Vetenarian
  ↓
  Doxycycline 250mg weekly
  Azithromycin 250mg weekly

Leptospirosis is the disease associated with
3R's : Rats
Ricefields
Rainfall

BORRELIA

✓ Causes : Relapsing fever, Lyme's disease
✓ Gram negative spiral
✓ Irregular spiral
✓ 8-30 μ long } thicker
✓ 0.3 - 0.5 μ broad
✓ Endoflagellate

Relapsing Fever

- Occurs due to Antigenic variation

2 types:

LOUSE BORNE Relapsing Fever	Tick borne Relapsing fever
EPIDEMIC Relapsing Fever	ENDEMIC Relapsing Fever
Causative organism ↓ Borrelia Recurrentis	Caused by 15 species: B. parkeri B. hermsii B. turicatae B. duttoni
Vector → Body louse	Vector → Soft tick (ORNITHODORUS)
Strict human pathogen No reservoir	Reservoir → Rodents
Mode of infection ↓ Feces inoculation Percutaneous/mucus membrane	Mode of infection ↓ Bite
Severe disease with 1-2 relapses	Milder diseases with 5-7 relapses
Mortality → 4-40%	Mortality - ~1%

Clinical features:

- Remitting Relapsing Fever
- I.P → 2-10 days
- Severe disease:
  - Jaundice
  - Hepatospleenomegaly
  - Lymphadenopathy
  - Hemorrhagic manifestation
  - Meningitis
  - Hemiplegia

Diagnosis:

1. Blood (Febrile stage)
   - (i) Wet mounts
   - (ii) Giemsa smear
   - (iii) Culture → BSK Medium (BARBOUR STOENNER KELLY)
   - (iv) NAAT
   - (v) Serology → Not useful (Because of Antigenic variation)

Treatment

- In louse borne relapsing fever

↓

Single dose of Tetracycline/Macrolide

- For tick borne relapsing fever

↓

Tetracycline/Macrolide

- In CNS involvement

↓

Intravenous penicillin
3rd generation Cephalosporin

LYME'S DISEASE

- ✓ MC Vector borne disease in USA
- ✓ Causative agent:
  - B. burgdorferi → In USA
  - B. afzellii } Asia
  - B. garinii
  - } EUROPE
- ✓ Vector : IXODID Species (Hard tick)
- ✓ Reservoir → Rodents, Deer
- ✓ I.P : 3-32 days

PRIMARY STAGE

↓

Erythema Chronic migrans (ECM)

(or)

Bulls Eye Rash

↓

Resolves spontaneously

SECONDARY STAGE

↓

Also known as Stage of Dissemination or Latest Great IMITATOR

- Generalised Lymphadenopathy
- 2° annular lesions (ECM like)
- Migratory arthralgia & Myalgia
- 5-10% → Meningitis
  - Lymphocytosis } In CSF
  - IgM, IgA, IgG }
  - → Carditis
    - ↓
    - Fluctuating A-V Blocks

After several years of latency leads to

TERTIARY STAGE

- also known as Stage of Persistence

(i) Chronic Symmetric polyarthritis
  MC in Knee

(ii) Encephalopathy
  Cranial nerve palsies
  Cerebellar Ataxia

(iv) Acrodermatitis chronicum atrophicans } Rare skin lesions

DIAGNOSIS

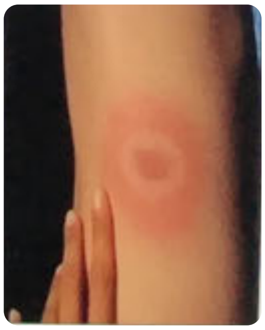
(i) Blood → In primary/secondary stage
↓
BSK Medium

(ii) Serology → MC used for diagnosis

(a) Screening → By ELISA/IFAT
(b) Confirmation → Western Blot

TREATMENT

- Drug of choice → Doxycycline 2wks to 2months
- CVS/CNS Involvement
  ↓
  IV Ceftriaxone
  (or)
  Penicillin for 2-4 wks



Chlamydia

- ✓ Gram negative coccobacilli
- ✓ Non-motile
- ✓ Obligate intracellular bacteria } Properties of virus
  Fitarable (through standard pore size of bacterial filters)
- ✓ Contain DNA & RNA } hence classified as BACTERIA
- ✓ Respond to antibacterial }
- ✓ Lack muramic acid in their cell wall
- ✓ Also known as :
  - * ENERGY PARASITES : ∵ They lack enzymes for ATP Synthesis
  - * BASOPHILIC VIRUSES : ∵ They form basophilic inclusion bodies
  - * PLT AGENTS : ∵ They cause 3 disease
    - PSITTACOSIS
    - LYMPHOGRANULOMA VENERUM
    - TRACHOMA

Obligate Intracellular bacteria:
- Chlamydia
- Rickettsia
- Mycobacterium leprae
- Treponema pallidium
- Spirillum minus → RAT BITE FEVER/SODOKU
- Tropheryma whipplei → WHIPPLE'S DISEASE

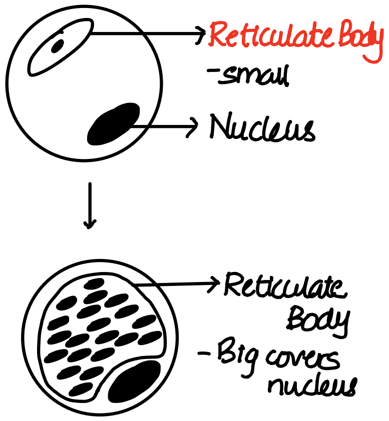
✓ All species of Chlamydia share same LPS Antigen but have species specific outer membrane proteins

LIFE CYCLE

- Biphasic

Elementary Body
↓
- Extracellular, infectious
- Metabollically inactive
↓
- Binds to receptor on cell membrane and becomes intracellular
- Inside Endosome, inhibits phagosome-lysosome fusion and forms RETICULATE Body

Reticulate Body
↓
- Intracellular, Non infectious
- Metabollically active
↓
- Undergoes binary fission
↓
- Produces 100-500 elementary bodies
↓
- Releasy by Lysis



* The reticulate body becomes large (binary fission) & covers the nucleus like a cloack, Hence the name CHLAMYDIA meaning CLOACK

STAINS

- Gram stain is not used
- Stains used:

Giemsa
Giminez
Casteneda
Machiavello
} Basophilic inclusion bodies ⊕

- C. trachomatis inclusion bodies contain

GLYCOGEN
⇓
IODINE STAIN CAN BE USED

CULTIVATION

- Non cultivable on cell free media
- So grown in:
  - (i) Guinea pig: method generally not used.
  - (ii) Yolk sac of Hen's Egg
  - (iii) Cell lines: That are irradiated/ antimetabolite added.

C. trachomatis + C. psittaci ⇒ Hela + McCoy
C. pneumonia ⇒ HeLa, McCoy, BHK (Baby Hamster Kidney), Vero

C. TRACHOMATIS

- Divided into 2 biovars

TRIC Biovars
↓
A, B, Ba, C ⇒ Trachoma
D ⟶ K ⇒ Genital Chlamydiasis

LGV Biovars
↓
L_1, L_2, L_3
⇓
Lymphogranuloma venerum, Proctocolitis

- A, B, Ba, C
  - causes trachoma ⟶ Kerato conjuctivitis
  - Inclusion bodies ⟶ HP bodies (Halber steadter Prowazek Bodies)

D-K Serovars

- strict human pathogen
- Reservoir: Human Urogenital Tract

MC bacterial STD
MC cause of urethritis (Non-gonococcal)
MCC of Ophthalmia neonaturum
MCC of Reactive arthritis

D-K (Males)

- Genital chlamydiasis in males
- I.P : 7 days
- 50% → Asymptomatic
- Cause urethritis, epidymitis, proctitis Reactive arthritis
- Symptomatic males ⟶ dysuria, urethral discharge

D-K (females)

- Genital chlamydiasis in females
- I.P: 7 days
- 80% → Asymptomatic
- Urethritis, Cervicitis: MC Manifestation
- Endometritis, salpingitis, P.I.D

- Fitz-Hugh Curtis syndrome
- Genital Chlymydiasis ⟶ MCC of P.I.D + Infertility
- They also cause →
  Ectopic Pregnancy
  Spontaneous abortion
  LBW infants
- Use of OCP's → ↑ the risk of Chlamydiasis
- In pre pubertal + post-menopausal ♀ → Vaginitis

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D-K in pregnant women
↳ Perinatal transmission
- → 50% risk → Inclusion conjuctivitis
- → 10% risk → Infant pneumonia

D-K → causes Swimming pool Conjuctivitis

DIAGNOSIS

(i) Specimens: Endourethral swabs, vaginal swabs, Cervical scraping, Conjuctival scraping, Urine sediment
(ii) Giemsa stain: Basophilic Inclusion Bodies
(iii) Antigen detection: Immunofluorescence
(iv) Cultivation: Yolk sac/ cell lines
(v) Serology: useful in invasive disease (PID/ Infant pneumonia)

MOST SENSITIVE METHOD:
Molecular methods: Probes (or) NAAT's

TREATMENT (D-K SEROVARS)

DISEASE	DRUGS
- Uncomplicated Genital Chlamydiasis	• DOC: AZITHROMYCIN (1g) single dose • Alternative: DOXYCYCLINE/ ERYTHROMYCIN/ OFLOXACIN for 7 days
- Complicated Genital Chlamydiasis	• DOXYCYCLINE/ ERYTHROMYCIN for 14 days
- Swimming pool Conjuctiva	• Single dose AZITHROMYCIN
- Neonatal (i) Conjuctivitis (ii) Pneumonia	ERYTHROMYCIN for 14 days.

LGV

- 3 serovars ⇒ L_1, L_2, L_3 → Strict human Pathogen
  ↓
  most common cause of LGV
- Sexually transmitted disease
- I.P: 3-30 days
- PRIMARY STAGE: Painless genital papule
  ↓
  Ulcer → Resolution in 7 days
- SECONDARY STAGE:
  - Regional Lymphadenopathy ⇒ Buboes
  - Inguinal Lymph node enlarged & matted with inguinal ligament below ⇒ GROOVE SIGN
  - Lymph nodes may ulcerate
  - Fever & Malaise
- TERTIARY STAGE: few month to years later
  - Genital Elephantiasis (due to block of lymph)

Eg: ESTHIOMENE → Elephantiasis of vulva

DIAGNOSIS

(i) Specimen: Swabs/Exudate, Lymph node biopsy/aspirate
(ii) Giemsa stain: MIYAGAWA BODIS/ GRANULO CORPUSCLES
(iii) Antigen detection: Immunofluorescence
(iv) Cultivation: Yolk sac/ cell lines
↳ most specific
(v) Molecular methods: most sensitive
(vi) FRIE'S TEST: Intradermal test using bubo pus extract. It's a hypersensitivity reaction not used due to ↑ false +ve values.

CHLAMYDIA PSITTACI

- zoonotic infection
- **Reservoir**: Psittacine birds (parrots)
  Non psittacine birds (pigeons)
- **Mode of infection**:
  - Inhalation of Aerosols of dried faeces/ body fluids of infected birds
  - Rarely by parrot bite
- Primarly occupational disease → Vetenarian/Pet shop owners

CHLAMYDIA PNEUMONIAE

- strict human pathogen
- Also known as TWAR Agent
- 1 serotype only
  ⇓ causes
  Pharyngitis, bronchitis, Atypical Pneumonia (~10% of community acquired Pneumonia)
- Chronic infection: ⇒ Atherosclerosis (CAD+CVD)
  ⇒ Asthma
- Treatment:
  DOXYCYCLINE/ERYTHROMYCIN x 3 weeks

PSITTACOSIS / ORNITHOSIS

→ **Atypical pneumonia** (U/L or B/L lower lobe infiltration)
→ C/O mildly productive/dry cough dyspnea
→ **Complication**: Septicemia leading to
1) Peri/ Myo /Endocarditis
2) Meningitis / Encephalitis
3) Hepatitis
4) Pyelonephritis

DIAGNOSIS:

(i) Specimen: Sputum, BAL (Broncho-alveolar Lavage), Lung biopsy
(ii) Giemsa stain:
LEVINTHAL COLE LILLIE BODIES (Inclusion Bodies)
(iii) Antigen detection: Immunfluorescence
(iv) Culture: BSL-III Lab (Biosafety Level)
(v) Serology: Most commonly used
(vi) Molecular methods: MOST SENSITIVE

TREATMENT:

Doxycycline / Erythromycin x 3 weeks

Rickettsia AND related genera

✓ Gram -ve, coccobacilli
✓ Obligate intracellular - EXCEPT BARTONELLA
✓ Arthopod transmitted - EXCEPT COXIELLA, BARTONELLA HENSELAE
NEO RICKETTSIA SENNETSIU
✓ Endothelial cell localize - EXCEPT EHRLICHIA & ANAPLASMA
✓ Fever, rash, vasculitis - C/F
Never seen in Q fever

STAINS:

- Giemsa
- Giminez
- Castaneda
- Machiavello

} Basophilic Inclusion Bodies

CULTIVATION:

Noncultivable on cellfree media
EXCEPT: Bartonella (Chocolate/BA)
a) Lab animals - Guinea pigs
b) Yolk sac - Hen's egg
c) Cell lines - HeLa, Hep 2

RICKETTSIA

Causes : a) Typhus group b) Spotted fever group (SFR's)

TYPHUS GROUP:

DISEASE	CAUSE	VECTOR	MOI	RESERVOIR	C/F
Epidemic typhus GOAL FEVER	R. prowazekii	Body louse	Mucous Membrane/ Skin breaks to louse feces	Man & flying Squirrels	Fever + Rash (starts at trunk) Myalgia, dellirium, Stupor or coma
Endemic typhus MURINE TYPHUS	R. mooseri (now typhi)	Rat flea & cat flea	Faeces or bites	Rodents	Milder ↳ latency not present

Epidemic typhus → can become latent in human → Reactivation disease (after many years) → called as BRILL ZINSSER DS RECRUDESCENT TYPHUS

SPOTTED FEVER GROUP

DISEASE	CAUSE	VECTOR	MOI	RESERVOIR	C/F
• RMSF (Most severe)	R. rickettsii	Hard tick	Bite	Rodents	Fever, rash (starts at palm & soles) Hemorrhages ⊕
• Indian/ Kenyan Tick typhus	R. conori	Hard tick	Bite	Rodents	Eschar Fever, Rash
• Boutteneuse fever	R. conori	Hard tick	Bite	Rodents	Eschar, fever, rash
• Rickettsial pox	R. akari	Liponyssoides mites	Bite	Mouse	Eschar, Fever, Rash self limited

RMSF = Rocky mountain spotted fever

SCRUB TYPHUS

- Also called as CHIGGEROSIS
- Caused by Orientia tsutsugamushi
- Vectors → Chiggers of mites (larval form)
  ↓
  TROMBICULID mites
- Reservoirs : Rodents & Birds
- Mode of infection : Bite of chiggers
- Clinical features :

  ESCHAR, fever, Truncal Rash, myalgia, generalised/ Regional lymphadenopathy
- MC Rickettsial infection in India : Scrub typhus

ZOONOTIC TETRAD:

Establish microfocus of infection when these things comes together

1) Orientsia tsutsugamushi
2) Chiggers of mites
3) Rodents & Birds
4) SCRUB VEGETATION

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DIAGNOSIS

1. Skin biopsy rash / Eschar → Giemsa staining → Basophilic inclusion bodies
2. Blood sample (Cultivation) → (a) Intra peritoneal guinea pig inoculation

↓

Scrotal inflammation
[called as Neil mooser / tunica Reaction]

(i) -ve in Scrub typhus
(ii) if Scrotal Necrosis → RMSF
(iii) +ve in R. typhi, R. conori, R. akari
(iv) -ve → only fever → R. prowazekii

(b) Yolk sac inoculation
(c) Cell line culture

3. Serology

a) Weil Felix reaction: Heterophile Agglutination (Tube agglutination test)

	OX-19 of P.vulgaris	OX-2 of P.vulgaris	OX-K of P.mirabilis
Epidemic Typhus	+ + +	+	−
Endemic Typhus	+ + +	+/−	−
Spotted fevers (except Rick pox)	+ +	+ +	−
Scrub Typhus	−	−	+

b) Species specific Ab's :
- IFAT or ELISA

TREATMENT :

DOC → Doxycycline
Alternative →
FQs,
Macrolides,
Chloramphenicol
SULPHONAMIDES → C/I

COXIELLA BURNETTII

- Biosafety level-III (BSL) pathogen
- Survives holder method.
- Survives in envionment as a highly resistant from resembling a spore
- PHASE (ANTIGENIC) VARIATION
  - ↳ Host → Phase i antigens
  - Cell lines → phase ii antigens
- Rash absent
- Causes Q fever (Query) - zoonoses

↓

milk & placenta ← Domestic animals [cows, sheep, goat]

- MOI → Raw milk, inhalation of aerosols Percutaneous

(i) Acute Q Fever :
- Fever, chills, Myalgia
- Resolves spontaneously

(ii) Severe Q Fever :
- ~ 5% of individuals
- Interstial pneumonia, Hepatitis

Risk factor for chronic :
- HIV
- Damaged / Prosthetic heart valves
- Chronic Renal failure

CULTURE -ve ENDOCARDITIS → Large nodular vegetation on heart valves.

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DIAGNOSIS

- Acute : i) Blood - shell vial culture
  Yolk sac culture
  ii) Serology - MC used
  - IgM +++,
  - 4 fold ↑ in Ab-titre
  - b/w acute & convalescent sera
- Chronic : marked ↑ titre of IgG ++

TREATMENT

Acute : Hepatitis or pneumonia
→ DOC DOXYCYCLINE x 2-4 weeks

Chronic :
DOXYCYCLINE + HYDROXYCHLOROQUINE
(or)
RIFAMPICIN
(or)
CIPROFLOXACIN
for 2-4 Years.

BARTONELLA

Bartonella henselae

- Reservoir : cats
- MOI : Cat lick on abraded skin, cat bites or scratch
- In NON-HIV patient → Cat scratch Disease
  - Papule/ Pustule + Ipsilateral Regional Lymphadenopathy
  - I.P → 3-10 days
  - Spontaneous resolution → 1-3 weeks

  DIAGNOSIS
  Biopsy of Papule → Warthin Starry Silver stain
  ↓
  Clumps of pleomorphic bacteria around endothelial cells
- IN HIV (+) Patients :
  → MC site → Skin → Bacillary Angiomatosis
  ↓
  Resembles Kaposi sarcoma

  Other sites :
  LIVER → Bacillary hepatitis
  SPLEEN → Bacillary splenitis
  MULTIPLE → Bacillary Peliosis

  DIAGNOSIS : same of Cat scratch dis.

  TREATMENT: ERYTHROMYCIN/DOXYCYCLINE

Bartonella quintana

Trench fever / 5-day fever / Shin bone fever

- Vector : Body louse
- MOI : Feces of louse
- Reservoir : Only man
- C/F : Fever, headache, truncal rash
  Myalgia, Arthralgia,
  Hepatosplenomegaly,
  Pre tibial pain
- Diagnosis : Serology (ELISA)
- Rx : Doxycycline

EHRLICHIA & ANAPLASMA

- intracellular Inclusion Bodies → Mullberry shaped k/a Morula
- E. chaffeensis : Human monocytic Ehrlichiosis
- A. phagocytophilia : Human granulocytic Ehrlichiosis
- Neorickettsia sennetsu : Fish ingestion
  ↓
  Sennetsu fever
- The vector of 1st two is HARD TICK

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